CRANFIELD UNIVERSITY

NETTRA SOMBOONKAEW

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN IMPORTED LITCHI FRUIT

CRANFIELD HEALTH
Plant Science Laboratory

Ph.D. THESIS
2010

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Ph.D. THESIS
Academic Years 2006-2010

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April 2010

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

It is important to determine the appropriate conditions for maintaining postharvest quality of litchi fruit after arrival from overseas markets. The present study, therefore, aimed to detail the effects of different storage conditions on physiological and biochemical changes in aril and pericarp tissues of imported litchi fruit.

Results from Chapter 4 were the first to fully detail the alterations of individual sugars, organic acids and total phenols in aril and individual anthocyanins in pericarp tissue as well as physiological changes under different storage temperatures in three cropping seasons. Results clearly demonstrated that higher concentrations of sucrose, malic, tartaric, and total phenolic contents were maintained at 5°C in aril and higher anthocyanins and total phenolic concentrations in pericarp during 13 days as compared with 8, 10, 13 or 20°C storage. Fruit treated at 5°C also had lower weight loss, brighter red pericarp colour and higher total soluble solids as compared against those fruit stored in other temperatures. A temperature of 5°C was therefore proposed for litchi distribution and storage, and acted as the basis of this study.

Relative humidity (RH) and vapour pressure deficit (VPD) in a storage environment are also important parameters affecting postharvest life. A new system using different glycerol solutions was employed to achieve defined RH levels in the present study. Although effects of RH on postharvest changes in litchi fruit have been described in previous works, the recent study is the first report detailing the effects of different and controlled VPD on litchi postharvest alterations. Low VPD was required to maintain quality of imported litchi during 9 days storage. In addition to reducing both weight loss and respiration rate, storage at 95-100 %RH and 5°C (VPD = 0.000-0.084 kPa) successfully remained high levels of sucrose and malic acid content in aril, and tartaric acid, cyanidin 3-rutinoside and mannose in pericarp tissue. It was therefore recommended that storage conditions for litchi should not only focus on maintenance of the cool chain, but should also consider controlling a VPD of ≤0.068 kPa to attain improved conservation of visual appearance.

Appropriate use of packaging materials can prolong shelf-life of assorted fruit and vegetables including litchi fruit. Imported litchi fruit were wrapped with either micro-

perforated polypropylene (PP), PropaFresh™ PFAM (PF), NatureFlex™ NVS, Cellophane™ WS or kept unwrapped prior to storage at 13°C for 9 days. Predictably, packed fruit retained better quality during storage as compared with unwrapped fruit. Each film tested resulted in an altered gas composition in the packages and thus affected postharvest quality. PF significantly decreased hydrolysis of sucrose in aril and retained higher cyanidin 3-rutinoside levels in pericarp. PF film also limited fruit weight loss and maintained sugar and organic acids concentration in both aril and pericarp.

Exogenous application of certain chemicals after harvest has been commercially used to control browning in litchi for many years. However, off-flavours may result and could potentially impact on consumer safety. These possible effects have enhanced demand for non-adulterated fruit on the market. Postharvest changes in pericarp and aril of non-adulterated and commercially-treated fruit as influenced by packaging films under different temperatures were detailed. Although commercially-treated fruit had higher aril organic acids and pericarp anthocyanins, sucrose hydrolysis in aril tissue was accelerated. The use of PF film at 5°C maintained higher sucrose and malic contents in aril tissue of non-adulterated and commercial litchi during 11 days as compared with unwrapped or PP regimes or 13°C storage. Results suggested that PF could replace PP as a new active film for the litchi industry and be a substitute for chemical treatment to maintain quality of litchi fruit.

ACKNOWLEDGEMENT

Foremost, I would like to express my sincerest gratitude to my supervisor Dr. Leon A. Terry for the continuous support of my Ph.D. research, for his patience, motivation, enthusiasm and immense knowledge. His guidance supported me in all the time of research and writing of this thesis and publications. I am grateful to Agricultural Research Development Agency (ARDA), Royal Thai Government for financial support. I would like to thank Minor Weir and Willis Ltd. and Agrexco Ltd. for supplying litchi fruit, and Innovia Films Ltd. for plastic films supplying. I offer my special thank for Dr. Anne-Do Bauchot (Agrexco) and Clare McKeown (Innovia Films) for their assistance. Dr. David Aldred and Dr. Deborah Rees are thanked for the suggestion. The kindest assistance and advice from Prof. Anthony K. Thompson and Dr. Gemma A. Chope are gratefully appreciated.

In the laboratory I have been assisted for many years in running the equipment by Allen Hilton, Balwinder Cook and Rosemary Burns. Prof. Naresh Magan gave useful guidance in the use of glycerol solution for setting relative humidity in Chapter 5. Charles Marshall and Dr. Sandra Landahl are thanked for statistical analysis advice. I have been blessed with a friendly, helpful, and cheerful group of Plant Science fellow students; Jordi Giné Bordonaba, Thanaraj Thiruchelvam, Marjolaine Meyer, Manuela Donetti, Katherine Downes, Maria Smyrniotaki, Sofia Foukaraki, Maria Kalogirou, Chandrakantha Mahendranathan and Niramon Suntipabvivattana.

Finally, I thank my parents, aunties and family for supporting me throughout all my studies.

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NOTATION

%	percentage
α	alpha
β	beta
<	less than
=	equals
>	greater than
°C	degree Celsius
µg	microgram
µL	microlitre
1-MCP	1-methylcyclopropane
a*	a colour scale: positive a* is red and negative a* is green colour
AAO	Ascorbic acid oxidase
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
ANOVA	Analysis of variance
APET	Amorphous polyethylene terephthalate
approx.	approximately
atm	Standard atmosphere
a_w	water activity
Berks.	Berkshire
BOC	British Oxygen Company
BOPP	Bi-axially oriented polypropylene
C*	chroma or colour intensity
C-T	Commercially-treated fruit
Ca	calcium
CA	Controlled atmosphere
ca.	circa (in Latin); approximately
CA.	California
$CaCO_3$	Calcium carbonate

cm	centimetre
cm^3	cubic centimetre
CO_2	Carbon dioxide
CPET	Crystalline polyethylene terephthalate
CU	Cranfield University
CV	Coefficient of variation
cv.	cultivar
cvs.	cultivars
d.f.	degree of freedom
Derbys.	Derbyshire
DW	dry weight
e.g.	exempli gratia (in Latin); for example
ELSD	Evapourative light scattering detector
EMA	Equilibrium modified atmosphere
et al.	et alii (in Latin); and others
EU	European Union
EVA	Ethylene vinyl acetate
EVOH	Ethylene vinyl alcohol
Exp.	experiment
FID	Flame Ionisation Detection
g	gram
g^{-1}	per gram
GAE	Gallic acid equivalent
GC	Gas chromatography
h	hour
h°	hue angle
h^{-1}	per hour
H_2SO_4	sulfuric acid
HDPE	High density polyethylene
Herts.	Hertfordshire
HPLC	High pressure liquid chromatography
IL.	Illinois

ISHS	International Society for Horticultural Science
kg	kilogram
kg^{-1}	per kilogram
kPa	kiloPascal
L	litre
L*	lightness
L^{-1}	per litre
LDPE	Low density polyethylene
LSD	Least significant difference
Ltd.	company limited
m	metre
M	molarity
m^2	square metre
m^{-2}	per square metre
m^3	cubic metre
m^{-3}	per cubic metre
MA.	Massachusetts
MAP	Modified atmosphere packaging
mg	milligram
Middx.	Middlesex
min	minute
min^{-1}	per minute
mL	millilitre
mm	millimetre
mol	mole
n	Number
N-A	Non-adulterated fruit
n/a	not applicable
N_2	Nitrogen gas
nm	nanometre
NVS	NatureFlex™ NVS
O_2	Oxygen gas

OA	Organic acid
OPP	Oriented polypropylene
P	probability
Pa	Pascal
Pa^{-1}	per Pascal
PAL	Phenylalanine ammonia lyase
PC1	direction of maximum variance in the dimentional cloud of points
PC2	direction of the next highest variance, subject to the constraint that it has zero covariance with PC1
PCA	Principal Components Analysis
Pd	Palladium promoted powdered materials
PE	Polyethylene
PET	Polyethylene terephthalate
PF	PropaFresh™ PFAM
pH	a measure of the acidity or alkalinity of the solution
pK_a	Acid dissociation constant
POD	Peroxidase
PP	Polypropylene
PPO	Polyphenol oxidase
PS	Polystyrene
PVC	Polyvinyl chloride
PVDC	Polyvinylidene dichloride
r	Pearson's product moment correlation coefficient
RH	Relative humidity
s	second
s^{-1}	per second
SEA	South East Asia
SiO_2	Silicon dioxide
SSC	Soluble solid concentration
ssp.	species
T	Temperature

TA	Titratable acidity
TBZ	Thiabendazole
TCD	Thermal conductivity detector
TSS	Total soluble solids
™	Trademark
UK	United Kingdom
UPVC	Unplasticised polyvinyl chloride
USA	United State of America
UV/VIS	Ultra violet visible
UVD	Ultraviolet detector
v/v	volume per volume
v/v/v	volume per volume per volume
viz.	videlicet (in Latin); namely
VP	Vapour pressure
VPD	Vapour pressure deficit
W. Sussex	West Sussex
w/v	Weight per volume
WS	Cellophane™ WS
XDB	extra densely bonded

CHAPTER ONE

Introduction

1.1 Project background

Whilst demand for fresh litchi in the world-wide market has increased moderately, the litchi industry is still confronted by two problems that lead to price deflation, decay and discolouration of fruit pericarp. Lack of adequate postharvest handling is the main reason for unmarketable quality. Unrefrigerated air storage of fresh litchi after harvest causes drying and browning of fruit pericarp. Although postharvest environmental controls including atmosphere, humidity, and temperature have been recently studied to limit the alterations in litchi fruit, growers and traders still face significant postharvest deterioration. Thus, the aim of this study was to detail the specific spatial and temporal physiological and chemometric changes in litchi fruit as affected by different storage conditions and modified atmosphere packaging, with specific emphasis on non-structural carbohydrates, non-volatile organic acids and phenylpropanoids in both aril and pericarp tissues.

1.2 Aim and objectives

1.2.1 Aim

The aim of this project was to determine the physiological and biochemical profile in imported litchi fruit under different storage conditions, and achieve the appropriate packaging for quality conservation and better shelf-life.

1.2.2 Objectives

- Determine the postharvest temporal and spatial changes in litchi as affected by temperature, relative humidity (RH), vapour pressure deficit (VPD), gaseous regimes and packaging films.

- Identify the optimum conditions for maintenance of litchi quality using chemometric analysis obtained from objective 1.
- Select appropriate packaging to achieve optimum conditions.

1.3 Thesis structure

There are eight chapters in this thesis. Chapter two explained the existing literature relating to litchi fruit including importance and current problems in litchi fruit production and trading and current postharvest physiological and biochemical profiles. Afterward, the recent effects of harvesting, postharvest handling and storage conditions on quality of harvested litchi fruit were detailed. Chapter three was the materials and methods for all experiments. This chapter described the preparation of samples and storage conditions, physiological measurement and biochemical analysis *viz*. weight, colour, respiration rate, CO_2 and ethylene concentration, sugars, organic acid, total phenols and anthocyanin.

Storage temperature has been reported as an important factors for prolonging fruit quality, despite there not yet been fully detailed the effect of storage temperature on postharvest physiological and biochemical changes in litchi fruit. Chapter four detailed the specific spatial and temporal physiological and biochemical changes in litchi fruit as affected by different storage temperatures (5, 8, 10, 13 and 20°C) with specific emphasis of respiration rate, weight loss, total soluble solids, sugar, non-volatile organic acids and total phenolic compounds in litchi aril tissues and together emphasis of colour and individual anthocyanins in pericarp tissues. Results from these experiments have been published as follow:

- Somboonkaew, N. and Terry, L.A. (2009). Effect of storage temperature on quality and taste-related compounds in imported litchi fruit. In: ISHS *1st International conference in postharvest and quality management of horticultural products of interest for tropical regions*. 20-23 July 2009. San Jose, Costa Rica. Oral presentation. (See Appendix D).
- A poster was presented at the Postharvest Unlimited 2008 – *Relationship between non-structural carbohydrate concentration and total soluble solids in*

litchi cv. Mauritius fruit stored at low temperature. 4-7 November 2008, Potsdam, Berlin, Germany. (See Appendix D).

There is still a lack of detailed information concerning the effects of other storage parameters on storage disorders in litchi. Although storage at elevated RH has been shown to maintain postharvest quality of litchi, the specific effects of a range of controlled vapour pressure deficit (VPD) levels (influenced by RH and temperature) on physiological and biochemical changes during storage have not been completely described. Chapter five described the explicit spatial and temporal physiological changes in imported litchi fruit cvs. Kom and Mauritus as affected by different storage VPDs (80, 85, 90, 95 or 100 % RH with 5 or 13°C), with particular emphasis on sugars and non-volatile organic acids in aril and pericarp tissue, and anthocyanins in pericarp tissue. Results from these experiments have been published as follow:

- Somboonkaew, N. and Terry, L.A. (2008). Deterioration of anthocyanins in litchi 'Kom'fruit stored under different relative humidity levels. In: *3rd International symposium on longan, lychee and other Sapindaceae family*, 25-29 August 2008, Fuzhou, China. Oral Presentation. (See Appendix D).

The results were submitted for publication in Journal of Agricultural and Food Chemistry on 23 December 2009.

- Somboonkaew, N. and Terry, L.A. Altered physiology and biochemistry of imported litchi fruit held under different relative humidity levels during storage. *Journal of Agricultural and Food Chemistry*. Submitted on 23 December 2009. (See Appendix D).

Modified atmosphere packaging (MAP) successfully prolongs shelf life of assorted harvested fruit and vegetables including litchi fruit. The previous works, however, have mostly been concerned with litchi pericarp browning e.g. degradation of anthocyanins. There is a lack of work reporting other biochemical changes in imported litchi fruit. Hence, Chapter six studied the physiological changes in litchi fruit as affected by different

packaging films but also elucidate the affect on sugars, non-volatile organic acids in aril and pericarp tissue and individual anthocyanins in pericarp. Results of this experiment have been published as follow:

- Somboonkaew, N. and Terry, L.A. (2009). Effect of packaging films on individual anthocyanins of non-acid imported litchi. In: *10th Controlled and modified atmosphere research conference*, 4-7 April 2009, Antalya, Turkey. Oral Presentation. (See Appendix D).

The results were accepted for publication in the Postharvest Biology and Technology on 23 January 2010.

- Somboonkaew, N. and Terry, L.A. (2010), Physiological and biochemical profiles of both aril and pericarp tissue from imported litchi fruit under modified atmosphere packaging. *Postharvest Biology and Technology*, vol. 56, pp. 246-253. (See Appendix D).

Although the influence of MAP on postharvest quality of litchi fruit was reported in chapter six, only non-acid treated and SO_2-free fruit have been experimented. Acid and SO_2 have been commercially used to minimise pericarp browning in harvested litchi fruit but the effect of these applications on physiological and biochemical changes have not fully described. Chapter seven detailed the influence of the combination of MAP and low storage temperatures on quality characteristic, CO_2 and ethylene production, aril sugar and organic acid and pericarp anthocyanin in acid and SO_2 treated fruit and acid- and SO_2-free fruit. Results from this work were submitted for publication in *Food Research International Journal*. Submitted on 2 February 2010.

- Somboonkaew, N. and Terry, L.A. Influence of temperature and packaging films on aril sugar in imported acid- and non-acid treated litchi fruit. *Food Research International Journal*. Submitted on 2 February 2010. (See Appendix D).

The results from previous chapters were generally discussed in chapter eight. This chapter proposes recommendations for future research and considers the postharvest handling quality of stored litchi fruit which minimise postharvest loss during supply chain.

CHAPTER TWO

Literature Review

2.1 Litchi economical importance and market trends

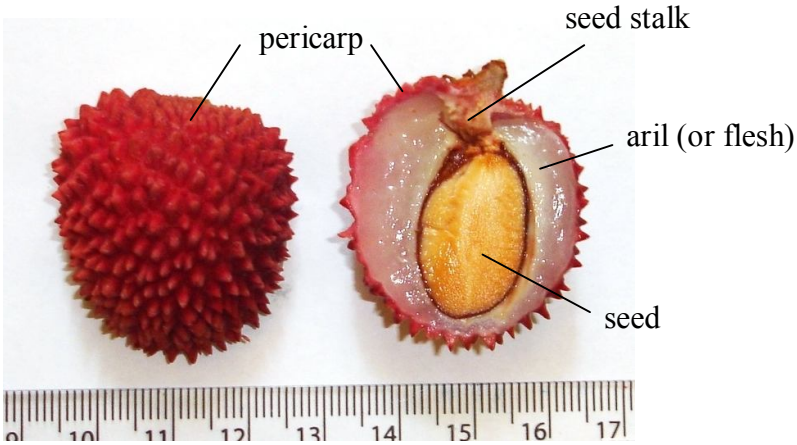


Figure 2.1. Litchi fruit cv. Kom from Thailand

A member of the Sapindaceae family, litchi (*Litchi chinensis* Sonn.) is a sub-tropical and tropical fruit and original to southern parts of China (Hai and Dung, 2002). China is the greatest producer and market for litchi fruit. In the past century, the litchi production area has been expanded to South East Asia (SEA), India, Taiwan, Australia, South Africa, the USA, Israel, South America, and the Mediterranean region with approximately 722,920 ha currently in production. Approximately 95% of the total litchi production is located in China, Vietnam, Thailand, India, Bangladesh and Nepal (Table 2.1.). Furthermore, because of high demand in domestic and increasing requirements in international trade, planting area of litchi has been expanded. In India, for instance, the growing area of litchi has risen from 1,330 ha in 1987 to 7,667 ha in 2003 but < 1% of total fresh produce is for export. Despite this, heightening popularity of exotic fruit on the global market and expanding of litchi production have manifested in litchi fruit becoming a principal fresh produce in international trade. Significant increasing of planting area in new commercial grower nations such as in Brazil and Mexico also emphasises growing interest in consumption of litchi fruit.

Table 2.1. Planting area and production of litchi

Country	Area (ha)	Production (tonnes)	References
Madagascar	3,000	20,000	Ghosh, 2001
Israel	300	2,000	Goren *et* al., 2001
Australia	1,500	5,000	Menzel, 2002
USA	240	1,000	Knight, 2001
China	588,000	1,280,000	Huang, 2002
Taiwan	12,000	108,000	Mitra, 2002
Viet Nam	30,000	50,000	Hai and Dung, 2002
Thailand	23,000	81,000	Sethpakdee, 2002
India	56,200	429,000	Singh and Babita, 2002
Bangladesh	4,800	12,800	Abu Baker Siddiqui, 2002
Nepal	2,380	14,000	Budathoki, 2002
South Africa	1,500	8,000	Huang *et al*., 2005

Most of the litchi fruit grown in China and SEA is destined for local regions. This is because of the enormous domestic demand. However, due to improvements in postharvest treatment for extending litchi shelf-life, export of the fruit to the EU, USA, and Middle East region has grown significantly.

In past decades, several litchi producers, particularly Israel and South Africa, have taken a very strong export focus. In 2000, over 50 % of fresh litchi crop from South Africa was exported to France, the UK, Germany, and the Netherlands (Huang *et al*., 2005). China has become a major exporter for litchi fruit for the past few decades. This largest producer obtained 6.7 million US dollar from export of 12,762 tonnes of fresh litchi fruit to the worldwide market in 1999 (Yi *et al*., 2002).

2.2 Current postharvest problem

Figure 2.2. Fresh pericarp (A) and discolouration and dry litchi pericarp (B) after stored at ambient temperature for 2 days

Whilst demand of fresh litchi in world-wide market has increased moderately, traders have confronted decay and discolouration of fruit pericarp, which lead to price deflation. Lack of sufficiently appropriate postharvest handling is the main reason for problems of unmarketable fruit (Figure 2.2.). Non-refrigerated air treatment after harvest of fresh litchi from orchard to local market causes dry and browning on fruit pericarp. For export, however, fumigation by SO_2 is applied for controlling pericarp browning and reducing postharvest decay. Excessive SO_2 can cause bleaching of the pericarp from reddish colour to yellow or pale green (Zauberman *et al*., 1990). Residue of this chemical also affects sensory quality of fruit aril, which results in a 'sulfur-like' odour and off-taste. Because of high residues of sulfur in the fumigated fruit, this treatment has been banned in several importing countries including the U.K. (Holcroft *et al*., 2005). Therefore, alternative treatments for controlling postharvest changes without toxic effects are preferred in response to concerns over food safety. For example, the efficacy of organic acid dips has been studied to minimise pericarp browning in recent years. Zheng and Tian (2006), for example, found that 2 and 4 mM oxalic acid could control postharvest browning of litchi fruit by inhibiting anthocyanin oxidation and degradation, increasing

membrane integrity, and maintain the relatively low peroxidase activity in litchi pericarp. Huang and Wang (1990) studied the effects of low storage temperatures and found that 5°C was the optimum for litchi cv. Hei Ye fruit with slight changes in *a** (negative values indicate green; positive values indicate magentaand) and *L** (0 indicates black and 100 indicates diffuse white) value, small decline in total soluble solids, and minimise moulding. Mahajan and Goswami (2004) also recommended temperature (2°C) is for litchi cv. Bombay fruit.

2.3. Postharvest physiology and biochemistry of litchi fruit

2.3.1. Cultivars: origin and characteristic

According to Leenhouts (1978), there are three subspecies in the litchi genus: *Litchi chinensis* ssp. *chinensis*; *Litchi chinensis* ssp. *javensis*; (Figure 2.3.) and *Litchi chinensis* ssp. *philippinensis*. However, the commercial litchi is spp. *chinensis*, which originated from Southern China and Northern Viet Nam.

spp. *chinensis* spp. *philippinensis*

Figure 2.3. *Litchi chinensis* Sonn.

There are many varieties of litchi in originated growing countries. New cultivars have been developed to provide desirable characteristics and higher productivity. However, most of the new cultivars have appeared as unexpected seedling or seedling assortments from recognised mother trees, which results in a restriction of hybridization (Menzel *et al*., 2005). Some cultivars have been called different names in different countries. The meaning of cultivar name mostly describes the prominent characteristics such as tree shape, leave colour, fruit colour, and fruit taste and juiciness (Table 2.2.).

Table 2.2. Characteristics of litchi in different cultivars

Cultivar	Meaning	Characteristics			Season
		Pericarp	Aril	Seed size	
Baila (Bah Lup, Dianbaibaila)	n/a	soft and thin with purple-red colour	juicy and sweet with shiny-white colour	large	May (China)
Baitangying (White sugar jar)	White Sugar Jar		Crisp and very sweet with shiny-white	large	May (China)
Chakrapad (Chacapat)	Emperor	thin and soft with deep red colour	big size, moderately juicy and a little sour in Thailand and high acid in Australia	large	July-August (Thailand)
Chenzi (Brewster, Floridian)	Chen Family Purple	thick and tough with murky purple-red colour	juicy, fragrant, juicy, vaguely sour with shiny-white colour	small	July (China) December (Australia)
Dazao (Tai So, Hong Huay, Mauritius)	Big Crop	thin with intense red colour	slightly tough and sweet with creamy white colour	large	June (China) December (Australia) May (Thailand) July-August (Israel) February (South Africa)
Feizixiao (Fay Zee Siu)	n/a	thin	firm, sweet, slightly fragrant	vary	June (China) October (Australia)
Guiwei (Kwai May Red, Kwai Mi)	n/a	thin with bright red colour	crisp, sweet and odorous with creamywhite colour	small	n/a
Heiye (Haak Yip, O-Hia, Baidum)	n/a	smooth with gloomy red colour	juicy, sweet and fragrant with creamy white colour	medium	June (China) April-May (Thailand) December (Australia)

n/a: not applicable

Table 2.2. Characteristics of litchi in different cultivars (cont.)

Cultivar	Meaning	Characteristics			Season
		Pericarp	Aril	Seed size	
Huaizhi (Wai Chee, Kim Cheng)	Cherished litchi	thick and tough with murky red colour	juicy, aromatic, slightly sour with shiny-white colour	small	June (China) February (Australia) May (Thailand)
Kom	Hunchback	thick with yellow-purple red colour	tough to fibrous and mild in taste	aborted	May (China) February-May (Thailand)
Lanzhu	Difficult to obtain	thin with yellow-purple red colour	juicy and faintly sour with creamy-white colour	aborted	June- July (China)
Luk Lai	n/a	crimson colour	n/a	small	n/a
Nuomici (No Mai Chee, No Mai Tsz)	n/a	thin and smooth	juicy, sweet and scented flavour	small	June (China)
Sah Keng	n/a	purple-red colour	soft and sweet with big size	small	n/a
Sanyuehong (Sum Yee Hong, Sun Yueh Hong)	Third Month Red	n/a	n/a	n/a	May (China)
Shuidong (Souey Tung, Yuanzhi)	n/a	thin and smooth with dreary purple-red colour	soft, juicy and sweet	medium	May-June (China) November-December (Australia)

n/a: not applicable

2.3.2. Fruit maturity

Litchi fruit must be harvested at the best possible visual appearance and eating quality (Underhill *et al*., 1997). Pericarp colour is frequently used for litchi harvesting index. Fruit pericarp should be bright red in colour without brown discolouration at harvesting. However, the maturity indices can be different for each cultivar, growing environment and region, and cultural practices. In addition, physiological characteristics of the litchi aril have tended to provide more guarantee as commercial maturity standards such as reaching the optimum range of total soluble solids (TSS):titratable acidity (TA) ratio (minimum ratio = 30; Kader, 2006) and freedom from defects and decay. Underhill and Wong (1990) also documented the relationship between the ratio of brix:acid and eating quality in nine litchi cultivars grown in Queensland, Australia. They found that overall the brix:acid ratio was 30-40 (calculated to give eating quality score of 5-6). Moreover, TSS of the aril increased with maturity to approx. 13-20 °Brix depending on cultivar, cultural practices and environment (Huang and Xu, 1983).

2.3.3. Respiration

Litchi is a non-climacteric fruit which has no substantial respiration peak after harvest. The wide range of values for respiration rate of litchi can be attributed to cultivar differences (Tongdee *et al*., 1982) as well as the continuing declined in respiration during storage. For instance, Puall and Chen (1987) reported that the respiration rate of litchi cv. Chenzi declined from 103 to 39 mg CO_2 kg^{-1} h^{-1} during storage at 22°C for 8 days. Besides, respiration rate of litchi cv. Calcutta fruit declined from 36.3 to 18.1 mg CO_2 kg^{-1} h^{-1} during 6 days storage at 25°C (Nagar, 1994). According to Chen *et al*. (1987), the respiration rate in litchi is high at room temperature and decreased markedly during cold storage. For instance, the respiration rate of litchi fruit (cultivar not stated) at 5, 10, and 20°C storage were 5-8, 10-15, and 25-40 ml CO_2 kg^{-1} h^{-1}, respectively (Kader, 2006). This is an important factor hampering the long-term storage of litchi. The high respiration rate may result in rapid consumption of nutrients, leading to a loss of taste.

2.3.4. Fruit composition

2.3.4.1. Sugars

The major sugars in litchi aril tissue are sucrose, fructose, and glucose (Huang *et al*., 1986 and Puall and Chen, 1987). There is considerable variation in the quantity of sugars between cultivars and different stages of maturity (Puall *et al*., 1984). Various levels of sugar in different litchi cultivars during maturation were reported by Wang *et al*., 2006 (Table 2.3.). Sucrose in full-mature aril tissue of litchi cv. Nuomici was found in greater concentration compare to glucose and fructose, whilst higher concentrations of glucose and fructose than sucrose were found in fully mature of litchi cv. Feizixiao fruit (Wang *et al*., 2006). Additionally, TSS in litchi aril tissue decreased during storage time (Tongdee *et al.*, 1982; Puall and Chen, 1987; Huang and Wang, 1990; Ketsa and Leelawatana, 1992; Nagar, 1994; and You *et al*., 1997), with absolute sugar content varying due to cultivar, maturity stage, and storage condition. According to Puall and Chen (1990), decreases in TSS in litchi cv. Hei ye were correlated with the decline of sucrose in aril tissue content and there were a slight initial decline in glucose and fructose. There have been fewer studies on the pericarp compared to the aril tissue. However, sucrose, glucose, fructose, mannose and galactose have been reported in pericarp (Yang *et al*., 2006).

Table 2.3. Sugar concentrations in the arils of eight litchi cultivars at maturity (Wang *et al*., 2006)

Sugar	Cultivar							
	Feizixiao	Xuehuaizi	Yuhebao	Sanyehong	Dahongli	Guiwei	Jixuili	Nuomici
Sucrose	5.9±0.10	5.0±0.19	7.4±0.18	9.2±0.28	9.4±0.44	12.2±0.22	11.7±0.06	11.7±0.17
Glucose	5.9±0.1	5.9±0.13	5.6±0.16	3.6±0.07	4.2±0.16	3.3±0.27	3.5±0.08	3.7±0.11
Fructose	6.3±0.08	5.8±0.13	5.7±0.21	3.3±0.06	4.1±0.13	3.5±0.21	3.6±0.05	3.8±0.12
Total sugars	17.4±0.23	16.7±0.24	18.7±0.24	16.1±0.43	17.7±0.41	19.0±0.25	18.8±0.11	19.2±0.36

*Unit: g 100 g^{-1} fresh weight

†Results are expressed as means ± SE (n = 5)

2.3.4.2. Acids

The concentration of total acids and titratable acidity in litchi aril decreases whilst pH increases during the fruit development (Puall *et al*., 1984; Huang *et al*., 1986). Nevertheless, Wang *et al*. (2006) reported that the concentration of total acids increased during the early stage of aril growth but reduced rapidly with aril maturation. Increase in pH and decrease of titratable acidity during storage time in litchi pericarp and aril tissue (Wu *et al*., 2001) can be ascribed to deterioration in malic, succinic acid (Wang and Chen, 1987) and ascorbic acid (Nagar, 1994).

Puall *et al*. (1984) and Puall and Chen (1987) reported that malic, succinic, and citric acid were the major organic acids (OA) in litchi aril tissue. Conversely, Wang *et al*. (2006) stated that the OA present in litchi aril tissue (cvs. Feizixiao, Xuehuaizi, Yuhebao, Sanyehong, Dahongli, Guiwei, Jixuili and Nuomici) were tartaric, malic, citric, and ascorbic acid whereas succinic acid was not found. Wu *et al*. (2001) reported that fresh mature litchi arils are a significant source of ascorbic acid (9.5-21.8 mg/ 100 ml) but the concentration decreased during storage. The fall in ascorbic acid content might be due to the oxidation by enzyme ascorbic acid oxidase (AAO), found in pericarp and juice (Lin, *et al*., 1988 and Nagar, 1994). According to Siriphanich (2006), this enzymatic oxidation converts ascorbic acid to the dehydroascorbic form (see section 2.3.5). The activity of AAO was related to the rapid decrease in the amount of the ascorbic acid, which is particularly associated with pericarp browning (Wu *et al*., 1995).

2.3.4.3. Phenolic compounds

Tannin, caffeic, vanillic, salicylic, gentistic and β-hydroxybenzoic acid, and 2-methyl resorcinol are the major phenolics in litchi fruit (Jaiswal *et al*., 1986). In addition, Zhang *et al*. (2006) reported that litchi pericarp (cv. Huaizhi) contained mainly flavol-3-ol monomers and dimers including (+)-catechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin, (-)-epicatechin 3-gallate, procyanidin B1, procyanidin B2, and procyanidin B4, which represent 87.0% of total phenolic compounds (Table 2.4.). Zhang *et al*. (2000) also suggested that gallic acid was the only hydroxybenzoic derivative found and no hydroxycinnamic derivatives were detected.

Contents of phenolics in litchi aril decreased during early fruit development and remained at a low concentration, which was less than 1 mg 100 g^{-1} fresh weight (Puall *et al*, 1984). Wu *et al*. (2001) documented that phenolics in both aril and pericarp tissue decreased during storage time. The total phenolic content was higher in the pericarp (1.4 mg 100 g^{-1}) than the aril (0.5 mg 100 g^{-1}) but the absolute concentration was cultivar dependent (Jaiswal *et al*., 1986).

Polyphenols can be degraded rapidly by polyphenol oxidase; PPO, which is also referred to as catechol oxidase, tyrosinase, catecholase or *o*-diphenol oxygen oxidoreductase). Phenols in litchi tissue are oxidised by PPO which results in the conversion of phenolic brown pigments (see section 2.3.5.). The PPO activity can be activated by moisture loss, mechanical injury, heat/chilling injury, and other stresses (Holcroft, *et al*., 1996; Siriphanich, 2006; Jiang *et al*., 2006). Moreover, peroxidase (POD) is another enzyme that involve in tissue breakdown and browning in litchi fruit. POD is determined as an index of plant senescence. Underhill and Critchley (1995) and Jiang and Fu (1999) documented that POD is an important aspect on browning of litchi pericarp. Recent research demonstrated that fruit cultivar (Underhill and Critchley, 1995), low relative humidity (Jiang and Fu, 1999), and storage conditions (Lin *et al*., 1988 and Huang *et al*., 1990) can influence the POD activity and thus the browning reaction. However, increase of POD activity was described as a response to plant tissue stresses (Siriphanich, 2006). Zauberman *et al*. (1990) also stated that POD is not main factor in determining rate of litchi pericarp browning.

Table 2.4. Phenolic compounds in litchi pericarp (cv. Huaizhi) (adapted from Zhang *et al*., 2000)

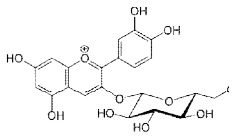
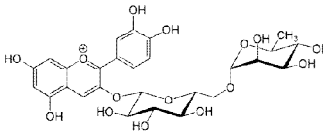
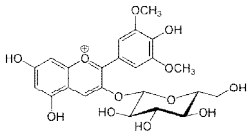
Phenolic compounds	Area (%)
(-)-Epicatechin	32.5
(-)-Epicatechin 3-gallate	23.3
Procyanidin B2	11.4
(-)-Epigallocatechin	10.9
Procyanidin B4	5.3
(+)-Catechin	1.6
(+)-Gallocatechin	1.1
Procyanidin B1	1.0
Gallic acid	0.4

2.3.4.4. Pigments

Flavonoids are the major pigment in mature litchi fruit (Jiang *et al*., 2006). The strongly coloured major flavonoids are anthocyanidins and anthocyanins (Salisbury and Ross, 1992; Hopkins and Huner, 2004). Anthocyanins differ from anthocyanidins by addition of various sugars, primarily at the 3-hydroxyl position (Table 2.5.). The pericarp of litchi fruit is initially green but changes to red with increasing pre-harvest maturity. The colour change is a result of a decrease in chlorophyll accompanied by an increase in anthocyanin synthesis. Synthesis of anthocyanin accounts for the red skin in mature litchi (Lee and Wicker, 1991 and Huang, 1995). These plant pigments are in general a cluster accountable for red, purple and blue colouration in flower, fruit, and leaves (Gross, 1987). They are water or alcohol soluble and situated in vacuoles in the mesocarp and epicarp of the lychee pericarp (Underhill and Critchley, 1994). However, once tissue turns brown, anthocyanins are soluble merely in organic solvent (Song *et al*., 1997). Anthocyanins can be degraded by enzymes such as PPO and POD (Markakis, 1982) that can contribute to browning of litchi pericarp. According to Gross (1987), in addition, these red to blue

pigments behaves like indicators in aqueous media-their structure and thus their colour vary with the pH (Figure 2.4.).

Table 2.5. The common anthocyanins found in litchi pericarp (adapted from Hopkins and Huner, 2004)

Anthocyanidin	Shade	+ Glycoside	= Anthocyanin	Structure
Cyanidin	Red-purple	3-glucoside	Cyanin	
Cyanidin	Red-purple	3-rutinoside	Keracyanin	
Malvidin	Deep-purple	3-glucoside	Oenin	

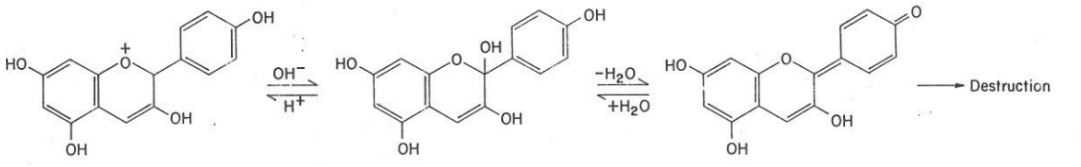


Figure 2.4. Structural changes of anthocyanins with pH (Gross, 1987)

2.3.5. Pericarp browning and its causes

The most significant postharvest disorder of litchi is pericarp browning. Browning can be caused by a wide range of different stresses such as non-climacteric conditions prior to fruit maturation (Sharma *et al*., 1986), disease (Huang and Scott, 1985; Jiang *et al*., 2002), desiccation (Scott *et al*., 1982; Underhill and Simon, 1993; Lin *et al*., 2002), fruit senescence (Huang and Wang, 1990), heat injury (Wong *et al*., 1991 and Jiang *et al*., 2002;), ethylene expression (Jiang *et al*., 1986) and chilling injury (Thongdee *et al*., 1982 and McQuire, 1997). Rapid postharvest pericarp desiccation and disease are by far the most frequent causes of browning (Underhill *et al*., 1997).

Browning in litchi pericarp has been mainly attributed to PPO, high POD activity (Zhang *et al*., 2005), ascorbic acid oxidation (Jurd, 1976; Jiang, 2000) and degradation of anthocyanins (Huang *et al*., 1990; Underhills, 1994; Wu *et al*., 1995; Jiang and Fu, 1999). The said factors associated with stresses such as wounds, postharvest conditions, and moisture loss disrupt cellular compartmentation, allowing PPO located in the chloroplasts and other plastids to react with phenolic substrates located in the vacuole, forming quinine and resulting in melanin (brown pigments) formation in the litchi pericarp (Macheix *et al*., 1990; Underhill and Critchley, 1995; Lin *et al*., 2002; Siriphanich, 2006), and this is combined with the coupled oxidation of the red anthocyanins by anthocyanase (Zhang *et al*., 2001; Holcroft *et al*., 2005). According to Jiang *et al*. (2006), anthocyanase hydrolyses anthocyanins to form anthocyanidin and which then may be oxidized by PPO, resulting in enzymatic browning. However, the role of anthocyanase in anthocyanins degradation still requires more research.

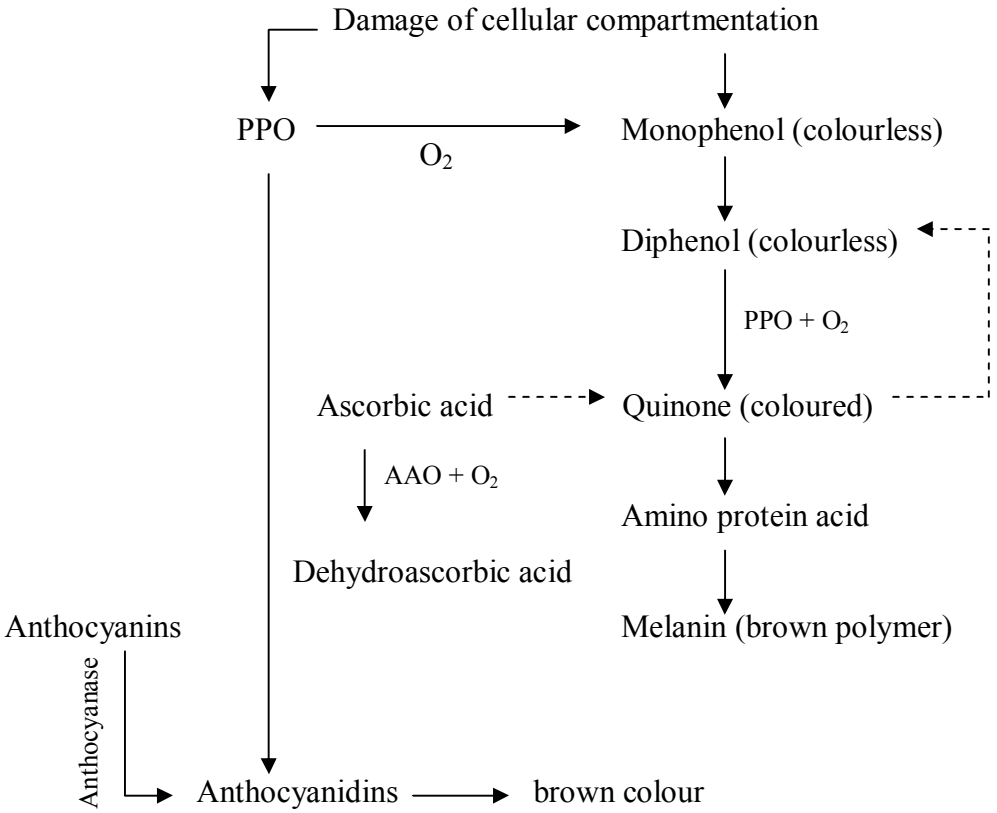


Figure 2.5. Enzymatic browning of litchi pericarp tissue

In addition, the colour of anthocyanins is dependent on environment, especially light, temperature, pH, metal ions, and phenolics (Gross, 1987; Holcroft and Mitcham, 1996). A rise in pH (in pericarp) converts the red flavylium cations into colourless carbitol or pseudobase. The colourless form allows expression of brown background colour which might appear. Desiccation, moreover, may increase the pH of cell sap hence the emphasis on reducing water loss to prevent browning. Micro-cracks in some cultivars of litchi fruit may expose the anthocyanins in the mesocarp to further desiccation and pathogen attack, leading to browning of the pericarp (Underhill and Simons, 1993).

2.3.6. Postharvest disorders and pathology

Litchi fruit is very susceptible to postharvest decay as a result of bacteria, yeasts, and other fungi. Many disease micro-organisms from harvest onwards are associated with postharvest decay of litchi (Table 2.6.).

Table 2.6. Major micro-organisms associated with postharvest decay of litchi fruit.

Organism	Reference
Alternaria sp.	Scott *et al*., 1982; Coates e*t al*., 1994; Sivakumar *et al*., 2008
Aspergillus sp.	Roth, 1963; Prasad and Bilgrami, 1973; Scott *et al*., 1982
Botryodiplodia sp.	Jiang *et al*., 2003
Cladosporium sp.	Scott *et al*., 1982; Sivakumar *et al*., 2008
Colletotrichum spp.	Scott *et al*., 1982; Coates *et al*., 1994
Fusarium spp.	Roth, 1963; Prasad and Bilgrami, 1973; Scott *et al*., 1982; Coates *et al*., 1994
Geotrichum candidum	Tandon and Tandon, 1975; Wind, 1992
Geotrichum ludwigii	Tsai and Hsieh, 1998
Lasiodiplodia theobromae	Prasad and Bilgrami, 1973; Scott *et al*., 1982; Coates *et al*., 1994
Penicillium spp.	Prasad and Bilgrami, 1973; Scott *et al*., 1982
Peronophythora litchi	Qu *et al*., 2001
Pestalotiopsis sp.	Prasad and Bilgrami, 1973
Phomopsis sp.	Scott *et al*., 1982; Coates *et al*., 1994
Rhizopus sp.	Scott *et al*., 1982
Yeast	Huang and Scott, 1985; Snowdon, 1990; Kadam and Deshpande, 1995; Sivakumar *et al*., 2008
Bacteria	Roth, 1963; Sivakumar *et al*., 2008

Micro-cracking in the pericarp was determined in some cultivars (Underhill and Critchley, 1992). The culticle layer of the thin pericarp consists of a partially formed cork layer, parenchyma cell, and stone cell with big intercellular spaces (Lin *et al*., 1991; Huang, 1995). Therefore, litchi fruit are predisposed to moisture loss. Fruit splitting depends on cultivar and is enhanced by desiccation, mechanical and pest induced damage and different humidity during late fruit development (Joubert, 1986; Holcroft and Mitcham, 1996). Softening of aril tissue is another postharvest disorder. Aril softening involves a loss of turgidity and translucency making the fruit becomes bland in taste. It is not associated to pericarp breakdown. It begins near the pericarp and appears to be more established at the end further from the stem (Holcroft and Mitcham, 1996).

2.4. Effect of harvesting and postharvest storage

2.4.1. Harvesting and grading

After reaching full maturity, litchi fruit are gathered by breaking or cutting the whole panicle and placed in plastic or bamboo baskets (Holcroft *et al*., 2005). Litchi fruit should be harvested with care and early in the day to reduce water loss and effects from sun heating. The fruit should be quickly cooled to remove field heat and transferred from orchard to packaging house to avoid the quality loss (Figure 2.7.). Rapid pre-cooling can achieve adequate results for later postharvest treatments (Thompson, 1998). For force-air cooling, fruit will be maintained in a high capacity cold room and take at least 12 hours to reach an effective commercial treatment (Watkins, 1990). To avoid moisture loss, the force-air cooling room should function at approx. 85% relative humidity. However, hydro-cooling has become a preferable procedure for litchi fruit in Australia, China, and Thailand due to lower risk of fruit desiccation (Jiang *et al*., 2004). Fruit are normally graded by market requirements, based on their size and weight (Jiang *et al*., 2003). In Thailand, grading includes classification freedom of blemishes, rots or insect damage, length of stalk, and freshness (Tongdee, 1994). For export, fruit are usually packed in plastic fibreboard carton, polystyrene boxes, or plastic crates (Holcroft *et al*., 2005) and supplied under cool chain distribution.

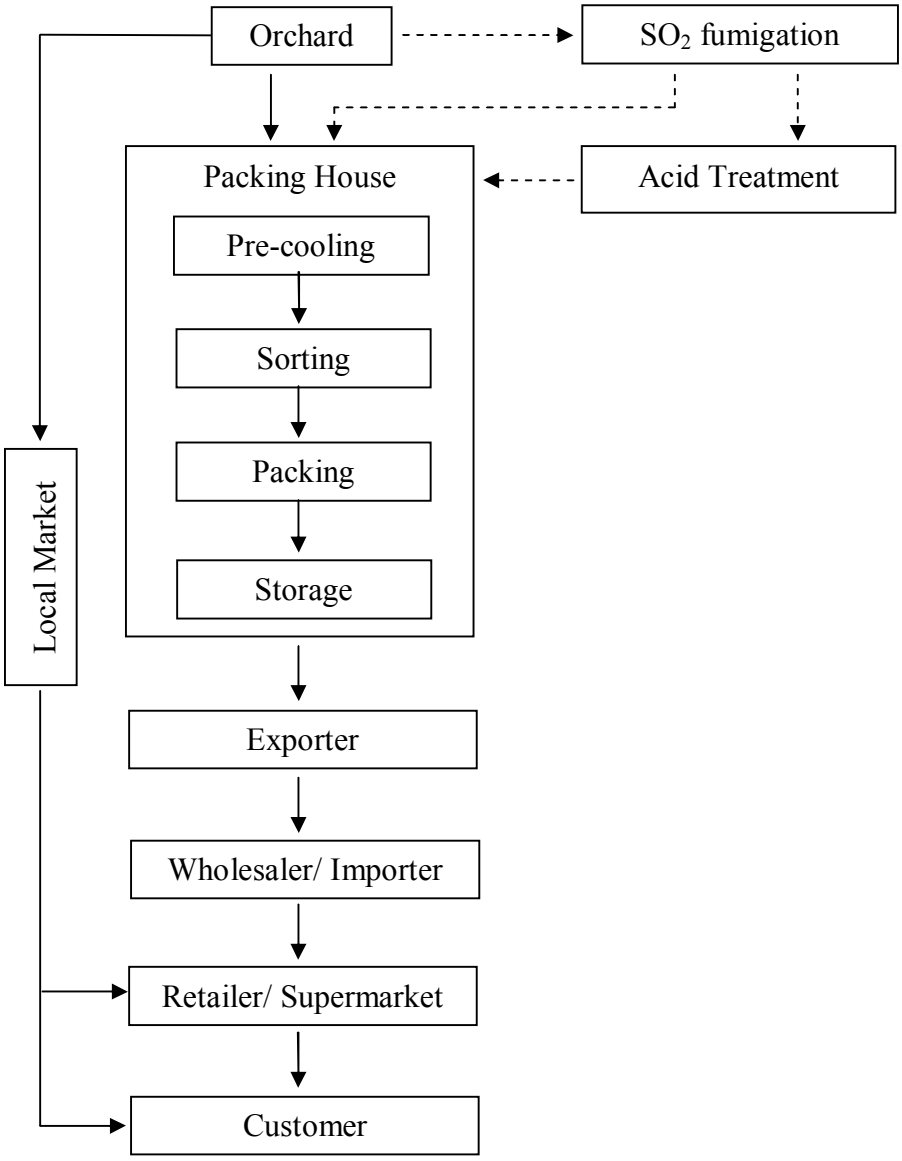


Figure 2.6. Litchi supply chain

2.4.2. Storage temperature

Refrigerated storage is one of the most effective tools for extending the postharvest life of fresh horticultural commodities. Low temperature does not only decelerate the fruit metabolism but also affects the rate of growth and spread of pathogens and decay.

For litchi fruit, pre-cooling and low temperature storage are common and have proved successful in prolonging postharvest life and quality. Pre-cooling eradicates vital heat from litchi fruit and is a significant predecessor to low temperature storage and transportation. Room cooling, forced-air cooling, hydro-cooling and vacuum cooling have been currently operated for litchi fruit. The effects of pre-cooling are revealed in Table 2.7. After pre-cooling, litchi fruit is stored at low temperature. The fruit can be stored at between 0 and 7°C for 14-60 days depending on the cultivar (Table 2.8.).

Table 2.7. Effects of pre-cooling on postharvest quality of litchi fruit

Cooling	Condition	Effect
Room/ Forced-air cooling	8-10°C, 12-14 h (Watkins, 1990) 3-5°C, 1 hr (Holcroft *et al*., 2005)	Fruit desiccation (< 95%RH) (Chen and Huang, 2001)
Vacuum cooling	-	Rapid cooling method but high water loss and browning (Chen and Huang, 2001)
Hydro-cooling	0-2°C iced-water, 2-3 h (Wang *et al*., 1996 and Lin and Chiang, 1988) 0-1°C iced-water, 12-15 min (Pornchaloempong *et al*., 1997)	-Fast cooling method but fruit may perish if packed wet (Chen and Huang, 2001) -Maintain fruit colour (Moreuil, 1973)

Table 2.8. Effects of storage temperature on harvested litchi fruit.

Cultivars	Storage temperature (°C)	Postharvest application	Storage Time (days)	Results						References
				Decay Occurrence (storage days)	% Browning (at last storage day)	TSS	Titratable acidity	Weight Loss	Other	
McLean's Red	2.0	BOPP packaging and hot water treatment	34	n/a	20	n/a	n/a	↑	Sensory: accepted	Sivakumar & Korsten,2006a
Bombay	2.0	CA storage	56	n/a	n/a	↑	↓	↑	Firmness ↑	Mahajan & Goswami, 2004
Huaizhi	4.0	TBZ dipping and LDPE wrapping	35	n/a	n/a	n/a	n/a	n/a	Phenolics ↓	Zhang *et al*., 2000
Wai Chee	5.0	Vapoure heat treatment	14	n/a	60-84	n/a	↓	n/a	Disease ↓	Jacobi *et al*., 1993
Hong Huay	5.5	H_2SO_4 dipping	15	n/a	20	↓	↓	n/a	-	Ketsa & Leelawatana, 1992
Seedless Late	0.0-3.0	Methyl-2-benzimidazole carbamate dipping	19	n/a	n/a	n/a	n/a	↑	-	Sandhu & Randhawa, 1992
Hei Ye	5.0	PE packaging	27	31	n/a	↓	↓	n/a	-	Huang & Wang, 1990
Hei Ye	2.0	Paper bag wrapping	30	20	90	↓	↓	↑	Res[1]., Anth[2].,SC[3]. ↓	Paull & Chen, 1987
Haak Yip	7.0	Bennomyl dipping and PVC film	40	n/a	46	↓	↓	↑	CI[4] (<7C,>30days)	Tongdee, *et al*., 1982

[1] Respiration,

[2] Anthocyanins,

[3] Sucrose,

[4] Chilling injury

2.4.3. Level of relative humidity in storage

High RH in the cold storage also plays an important role to prolong postharvest life of many fresh produce types. Jiang and Fu (1999) and Kaewchana *et al*. (2006) studied the influences of RH (range 50-90 %RH) on pericarp browning of litchi fruit cv. Huaizhi and cv. Hong Huay, respectively. Both studies reported that litchi stored at 90 %RH showed the lowest browning scale and pericarp desiccation, slightest changes in a* and L* values, smallest decrease in anthocyanin and phenolic contents, slowest increase of PPO and minor activity of phenylalanine ammonia lyase (PAL), followed by the fruit stored at 80, 70, 60 and 50 %RH, respectively. High storage RH of between 80 and 98% was found to prolong quality of litchi. However, higher humidity storage without adequate ventilation can cause water soaking and accelerate decay in litchi fruit (Jiang *et al*., 2004). Other approaches used are summarised in Table 2.9.

Table 2.9. Storage condition for litchi fruit.

Cultivar	Storage condition	Storage time (days)	Reference
McLean's Red	95%RH with biorientated polypropylene at 2°C	34	Sivakumar and Korsten, 2006a
Heiye	95%RH with CA conditions at 3°C	42	Tian *et al*., 2005
Bombay	92-95%RH at 2°C 40-71%RH at 28-36°C	28 8	Mahajan and Goswami, 2004
Hong Huay	80%RH with polyethylene film liner at 5°C	12	Ketsa and Leelawatana, 1992

2.4.4. Gas composition in storage

Low temperature and high RH, operated with other postharvest treatments such as acid dipping, are documented to be successful in prolonging the shelf-life of litchi fruit (See 2.3.1.and 2.3.2.). Postharvest life of litchi can be further extended by using CA and/ or modified atmosphere (MA) techniques in which storage atmosphere is accurately controlled to low O_2 and high CO_2 with cold storage. Low concentration of O_2 and high CO_2 (>0.03%) can decelerate the fruit respiration and metabolism (Thompson, 1998, and Siriphanich, 2006). This reduces fruit deterioration and results in shelf-life extension. Controlled and modified gas compositions in storage have been reported to decrease browning and decay; maintain ascorbic acid, TSS, titratable acidity; and lengthen the storage life of litchi fruit (Table 2.10).

High O_2 atmosphere, additionally, has been studied to extend storage life and maintain postharvest quality of litchi. According to Duan *et al*. (2004), exposure to 100 % O_2 was effective in preventing pericarp browning of Huaizhi litchi after 4 days storage at 25°C and 80-85 %RH. They indicated that high levels of ATP, ADP and energy charge of litchi kept in pure O_2 containers may contribute to retain membrane integrity and thus reduce decompartmentation of enzymes and substrates resulting in pericarp browning. However, Techavuthiporn *et al*. (2006) stated that storage life of litchi cv. Hong Huay preserved in 50 and 70 % O_2 chambers (at 4°C, 90-95 %RH) was 28 days or 8 days longer than fruit kept in 90 % O_2 in air chambers. They also found that total anthocyanin content decreased significantly in 90 % O_2 corresponding with the increased rate of pericarp browning after 16-20 days storage.

2.5. Packaging

Various postharvest chemicals have been applied for maintaining the quality of fresh produce. SO_2, in particular, can retain the marketable quality of litchi fruit by suppressing pathogens, insect, and physiological browning (Underhill *et al*., 1997). However, SO_2 residues can bleach the pericarp and also lead to the production of off-flavours in the fruit (Holcroft *et al*., 2005). There are also increasing concerns

Table 2.10. Effects of gas composition in storage**.**

Cultivar	Storage condition				Other Treatment	Storage time (days)	Effect	Reference
	O_2 (%)	CO_2 (%)	Temp. (°C)	Relative humidity (%)				
Heiye	5	5	3	95	-	42	Delay anthocyanin degradation, reduce fruit decay, decrease total phenol content, control pericarp browning, maintain good flavour	Tian, *et al.*, 2005
Huaizhi	-	-	20	95-100	Pure N_2 exposure for 3 or 6 h (Anoxia)	6	Decrease browning, control decay, maintain amount of TSS, titratable acidity and AA[1]	Jiang, *et al*., 2004
Bombay	3.5	3.5	2	92-95	-	56	Inhibit change of *a** value, low weight loss, slight changes in acidity and CA content, small changes of TSS, sensory acceptation	Mahajan and Goswami, 2004
Huaizhi	3-5	3-5	1	90	TBZ[2] dip	30	Retain red bright colour in pericarp, small change of AA[1] , low weight loss	Jiang and Fu, 1999
Mauritius	3	5	5	n/a	-	22	Rational flavour and texture	Vilasachandran *et al*., 1997
Mauritius	n/a	15	5	n/a	-	22	Off flavour and dull grey aril	Vilasachandran *et al*., 1997

[1] Ascorbic acid ,

[2] Thiabendazole

of SO_2 residues on consumer health. Residues and application of this chemical on food have been limited in many countries e.g. USA, EU, and Japan (Holcroft and Mitcham, 1996). Alternative techniques to control postharvest diseases and browning have been sought to replace SO_2 treatment.

Packaging technology is a method which can maintain postharvest quality of horticultural commodities. MAP effectively extends shelf-life of assorted fresh produce including litchi fruit (Holcroft and Mitcham, 1996 and Jiang *et al*., 2006). Similar to CA storage (see 2.4.4. gas composition in storage), MAP relies on respiration rate of fresh produce, concentration of O_2 and CO_2, and temperature management (Siriphanich, 2006 and Thompson, 1998). MAP relies on the thickness and permeability of the polymeric film (Roming and Mir, 2000 and Erkan and Wang, 2006). This element plays a significant role to establish new atmospheric conditions in the package and lead to postharvest life prolongation.

Polymeric film is the main packaging material used for MAP. Several types of polymeric film are used for fruit and vegetable packaging. Each type of film has different properties (Table 2.11.), which is suitable for different types of produce characteristics. Polyethylene (PE) and polyvinyl chloride (PVC) film are commercially selected for fresh produce because of their properties and low cost. Both polymeric films (Table 2.8.) were reported to control pericarp browning and extend shelf-life of litchi fruit efficiently (Tongdee *et al*., 1982; Huang and Wang, 1990; Ketsa and Leelawattana, 1992; Zhang *et al*., 2000 and Chaiprasart, 2005). Under inappropriate MAP conditions, however, an increase in ethanol and acetaldehyde from litchi fruit can result in anaerobic respiration (Pesis *et al*., 2002), leading to unacceptable taste, odour and flavour.

Table 2.11. Properties of polymeric film (Greengrass, 1998)

Film	Water vapour transmission ($g.m^{-2}$ per 24 h at 38°C, 90% RH)	Gas transmission rate ($cm^3.m^{-2}.atm^{-1}$ $24h^{-1}$ for 1 ml film at 25°C)		
		Oxygen	Nitrogen	Carbon dioxide
LDPE	18	>800	2800	42000
HDPE	7-10	2600	650	7600
PPcast	10-12	3700	680	10000
OPP	6-7	2000	400	8000
OPP coated with PVDC	4-5	10-20	8-13	35-50
Ionomer	25-35	6000	-	6000
EVA	40-60	12500	4900	50000
UPVC	30-40	150-350	60-150	450-1000
Plasticized PVC	15-40	500-30000	300-10000	1500-46000
PVC/ PVDC copolymer	1.5-5.0	8-25	2-2.6	59-150
EVOH	16-18	3-5	-	-
PS oriented	100-125	5000	800	18000
APET	40-50	110-130	-	-
CPET	Permeabilities change according to degree of crystallinity. For each 1% increase there is a 1.5% decrease in transmission rate			
PET oriented	25-30	50-100	15-18	180-390
PET oriented and coated PVDC	1-2	9-15	-	20-30

LDPE: low density polyethylene, HDPE: high density polyethylene, PP: polypropylene, OPP: oriented polypropylene, PVDC: polyvinylidene dichloride, EVA: ethylene vinyl acetate, EVOH: ethylene vinyl alcohol, PS: polystylene, UPVC: unplasticised polyvinyl chloride, APET: amorphous polyethylene terephthalate, CPET: crystalline polyethylene terephthalate, PET: polyethylene terephthalate

Although the accumulation of water vapour results in high relative humidity in the package which limits pericarp desiccation, excessive moisture can accelerate growth of spoilage organisms (Erkan and Wang, 2006 and Mahajan *et al*., 2007). The properties of polymeric films have been developed to match to fresh produce characteristics. In the recent past, gas permeability of polymer film has been improved to meet compulsory conditions such as lowest aerobic respiration rate and desirable level of CO_2 and water vapour. Three areas of film development have been examined: novel chemistry, intentional additives and perforation technology.

High permeability films are usually merged from two to three different polymers. Each polymer has a particular purpose such as strength, gas permeability and transparency and can be laminated with other polymers to accomplish a desired attribute. Polymeric film can also be integrated with inert inorganic substance such as $CaCO_3$ and SiO_2 (Nicholson, 2006) to create a micro-porous film. The gas permeability can be directed by setting the filler content, particle size of the filler, and degree of stretching. Several studies reported the influence of size and quantity of pore on gas permeability of MAP and quality of fresh fruit. For instance, Pesis *et al*. (2002) reported that the laminated PE bags were perforated either twice with a 21G needle (as micro-perforated film) or four times with 0.6 cm (as macro-perforated film) before packing litchi fruit cv. Mauritius. They found a higher accumulation of CO_2, acetaldehyde, and ethanol in litchi stored in micro-perforated film than macro-perforated MAP after 31 days storage at 2°C (28 days) and 20^{o}C (3 days). There was a greater concentration of CO_2, acetaldehyde, and ethanol which inhibited fungal growth and thus limited decay development. Micro-perforated film, also, maintained a healthier pericarp appearance due to higher relative humidity in the package. However, high amounts of acetaldehyde and ethanol in micro-perforated bag resulted in undesirable flavour. Similar effects of perforated film were found with various horticultural produce such as citrus (Porat *et al*., 2004) and sweet cherry (Alique *et al*., 2003). To achieve more efficient MAP, an appropriate ratio of fresh produce to polymeric film permeability has been determined. Study of the processes of gas exchange and response of fresh produce in MAP system, has demonstrated in the current past.

The design of a MAP system aims to characterise conditions which will form the atmosphere most appropriate for the prolonged storage of fresh produce. This can be completed by matching the permeation rate of polymeric film for O_2 and CO_2 with the

respiration rate of the produce (Cameron *et al*., 2001). To compute the required O_2 and CO_2 permeability for fresh fruit, an optimal gas concentration in the MAP is created. This should be based on the steady-state respiration rate whereas in MAP the respiration rate changes as the atmosphere is modified (Hayakawa *et al*., 1975 and Cameron, 2001). Several studies have reported on the use of modelling and MAP systems. Mahajan *et al*. (2007) clarified the user-friendly software for design of MAP for fresh and fresh-cut produce. They found that the software could select suitable packaging materials (including polymeric film, macro- and micro-perforated film) and discover the quantity of product to be packed relative to the area of the film that should be available for gas exchange.

Another factor relevant to successful MAP is storage temperature (Cameron *et al*., 1995 and Mahajan *et al*., 2007). This is due to the difference in rate of change of permeability and respiration rate with temperature, a film that generates a complimentary atmosphere at the optimal storage temperature may cause excessive increase of CO_2 and reduction of O_2 at higher temperature, a situation that could lead to metabolic disorders (Beaudry *et al*., 1992; Cameron *et al*., 1994).

Storage temperature is never constant in the supply chain of fresh produce (Jacxsens *et al*., 2000). The temperature determines respiration of fresh produce and O_2 permeability of packaging film, variable temperatures result in changes of internal O_2 and CO_2 concentration of equilibrium modified atmosphere (EMA) packaged fresh produce. Thus, the design of EMA packages have been improved by adding mathematical models relating the effect of temperature and O_2 and CO_2 concentration on respiration. This can result in a better understanding of the correlation between produce type, produce weight, temperature, O_2 and CO_2 dependence of fresh produce respiration, film type, package area, and temperature dependence of film permeability for O_2.

CHAPTER THREE

Methodology

3.1 Sample preparation

3.1.1. Experiments 1-3 (Chapter 4)

Litchi cv. Mauritius fruit originating from Israel (Agrexco Ltd.) for Experiments (Exp.) 1, 2, and 3 were harvested on 28 June 2006, 24 July 2006, and 14 August 2006, respectively, and transferred to the packinghouse (Milopri, Western Galilee, Israel) within an hour in covered but non-refrigerated lorries. Fruit was subsequently treated with sulphur (1 g sulphur for 1.5 kg of fruit) in a purposed-build tent with 20 % extra volume than the volume of fruit at 20°C for 30 min (within 4 h of harvest) and pre-cooled at 1°C for 8 h. After this, litchi fruit was air-freighted to the UK (Exp. 1 and 3) and by sea-freight (Exp. 2). Upon arrival in the UK, fruit from Exp. 1, 2, and 3 was transported by refrigerated lorry to Minor Weir and Wills Ltd. (Birmingham, UK) and stored at 1°C on 3 July 2006, 3 August 2006, and 20 August 2006, respectively. Fruit was then transported to the Plant Science Laboratory (Cranfield University; CU) by car one day later and pre-cooled at 5°C for 2 h before being sorted for uniformity. Thus, fruit for Exp. 1, 2 and 3 were 6, 11 and 6 days old from harvest, respectively. All fruit was not pre-treated with acid.

Each experiment was arranged as a completely randomised design and the samples were taken randomly. Fruit was separated into either 4 (Exp.1) or 3 sets (Exp.2 and 3) and then stored at 5, 8, 10, and 13°C (Exp.1) or 5, 13 and 20°C (Exp.2 and 3) with approximately 65 % relative humidity (RH). The storage temperatures were monitored using Tiny Tag Ultra 2 data loggers (Gemini Data Logger, W. Sussex, UK). For each experiment fruit was divided into two samples for destructive physiological analysis and non-destructive respiration measurement.

Individual fruit from Exp. 1 (n = 432), 2 (n = 324) and 3 (n = 324) were weighed and then divided equally into commercial polypropylene (PP) plastic punnets: 140 × 115 mm (Nicholas Ltd., Derbys., UK). According to industry practice in the UK, each punnet

was then individually placed in a micro-perforated PP plastic bag (Nicholas Ltd.): 150 × 200 mm with 25 μm thickness and heat-sealed by hand operated heat sealer (Hulme Martin Ltd., Surrey, UK). The packed fruit (6 fruits per punnet) was studied over a period of 13 days at day 0, 1, 3, 7, 10, and 13.

Table 3.1. Experiments in the thesis

Experiments	Cultivars	Origin	Chemical applications	Outturn (days of storage)	Treatments	Measurements
Exp. 1 (n = 432)	Mauritius	Israel	SO_2, non-acid dip	0, 1, 3, 7, 10, 13	Storage Temperature: 5, 8, 10, 13°C	-Weight: individual fruit weight loss, pericarp and aril dry matter and moisture content -Respiration rate (Exp. 1-5) -Pericarp colour (individual fruit) -Total soluble solids (individual fruit) -CO_2 and ethylene concentration (Exp. 6-7) -HPLC analysis -Sugars: aril and pericarp (Exp.4-6) and only aril (Exp.1-3, 7) -Organic acids: aril and pericarp (Exp.1-7) -Pericarp anthocyanins (Exp.1-7) -Total Phenols: aril and pericarp (Exp.1-3)
Exp. 2 (n = 324)	Mauritius	Israel	SO_2, non-acid dip	0, 1, 3, 7, 10, 13	Storage Temperature: 5, 13, 20°C	
Exp. 3 (n = 324)	Mauritius	Israel	SO_2, non-acid dip	0, 1, 3, 7, 10, 13	Storage Temperature: 5, 13, 20°C	
Exp. 4 (n = 300)	Kom	Thailand	SO_2, non-acid dip	0, 1, 3, 6, 9	Relative Humidity: 80, 85, 90, 95, 100 % Storage Temperature: 5, 13°C	
Exp. 5 (n = 300)	Mauritius	Israel	SO_2, non-acid dip	0, 1, 3, 6, 9	Relative Humidity: 80, 85, 90, 95, 100 % Storage Temperature: 5, 13°C	
Exp. 6 (n = 450)	Mauritius	Israel	Non-SO_2, non-acid dip	0, 2, 4, 6, 9	Packaging films: Perforated polypropylene, PropaFresh™ PFAM, NatureFlex™ NVS, Cellophene™ WS Storage temperature: 13°C	
Exp. 7 (n = 864)	Mauritius	Israel	1). Non-SO_2, non-acid dip 2). SO_2 and citric acid dip	0, 3, 7, 11	1). Non-SO_2 and acid: Perforated polypropylene, PropaFresh™ PFAM; Storage Temperature: 5, 13°C 2). SO_2 and acid: Perforated polypropylene, PropaFresh™ PFAM; Storage Temperature: 5, 13°C	

3.1.2. Experiments 4-5 (Chapter 5)

Litchi cv. Kom fruit, grown in Samutsongkram province was exported by the River Kwai International Food Industry Ltd. (Bangkok, Thailand) to the UK (Minor Weir and Wills Ltd.). Fruit was harvested (fruit core temperature = 29°C) on 14 April 2007, hydro-cooled (fruit core: 6°C) and transported to the packhouse by refrigerated lorry (*ca*. 12°C). As per standard practice, fruit was treated with SO_2 within 7 h of harvest before being stored at 2°C and transported to the airport by refrigerated-truck within 24 h of harvest. Israeli cv. Mauritius litchi fruit grown in Western Galilee, was imported to the UK by Agrexco Agricultural Export Ltd. (Middx., UK). Fruits were harvested on 17 July 2007 and treated with SO_2 within 5 h of harvest. Afterward, fruit was pre-cooled at 1°C for 8 h and air-freighted to the UK. Upon arrival in the UK, litchi cv. Kom fruit was transported by refrigerated lorry to Minor Weir and Wills Ltd. and cv. Mauritius to the Agrexco (Middx., UK) and stored at 1°C. Importantly, both litchi cultivars were not pre-treated with acid as use of postharvest acid dip is becoming increasingly less desirable for UK consumers. Fruit cvs. Kom and Mauritius were transported to Cranfield University within 2 h, and thus arrived at the laboratory within 4 and 6 days of harvest, respectively. Fruit were sorted for uniformity of size and freedom from defects.

Both experiments were arranged as randomised complete blocks. Thai (n = 300) and Israeli fruit (n = 300) were separated into 10 groups. Each group was divided equally into 6 PP plastic punnets: 115 × 140 mm (Nicholas Ltd.). Each punnet (5 fruits per punnet) was then individually placed in a micro-perforated PP plastic bag (Nicholas Ltd.): 150 × 200 mm with 25 μm thickness as per standard commercial practice in the UK and heat-sealed using a hand-operated heat sealer (Hulme Martin Ltd.). Fruit punnets were stored at 80, 85, 90, 95 and 100 % RH in 25 L polyethylene plastic boxes (HK-Plastics BV, Oldenzaal, The Netherlands) for 9 days at either 5 or 13°C.

RH was controlled in containers according to Pateraki *et al*. (2007) with modification. Regular air was bubbled through 500 ml glycerol solution (116, 82, 53 and 24 % (v/v) glycerol in distilled water to achieve 80, 85, 90 and 95 %RH, respectively) or pure distilled water (for *ca*. 100 %RH) for 3 min at 30 min intervals for 9 days and exhausted continuously at 3 L min^{-1} flow rate using a gas mixing blender (Signal Series 850, Signal Instrument Ltd., Surrey, UK) (Figure 3.1). The experiments only started once

RH had equilibrated for 4 days (96 h). After boxes were opened and fruit put inside, desired RH levels returned within minutes. RH in each container was monitored using Tiny Tag Ultra 2 data loggers (Gemini Data Logger). The vapour pressure deficits (VPD) was then calculated using the following equation (1) (Thompson, 1998) where the vapour pressure at saturated condition (VP(s); (2)) was based on measured temperature (T in °C) and the vapour pressure in the storage (VP; (3)) was based on the controlled RH (%).

$$VPD = VP(s) - VP \quad (kPa) \tag{1}$$

$$VP(s) = 0.6108\, e\left(\frac{17.27 \times T}{T+265.5}\right) \tag{2}$$

$$VP = \frac{VP(s) \times RH}{100} \tag{3}$$

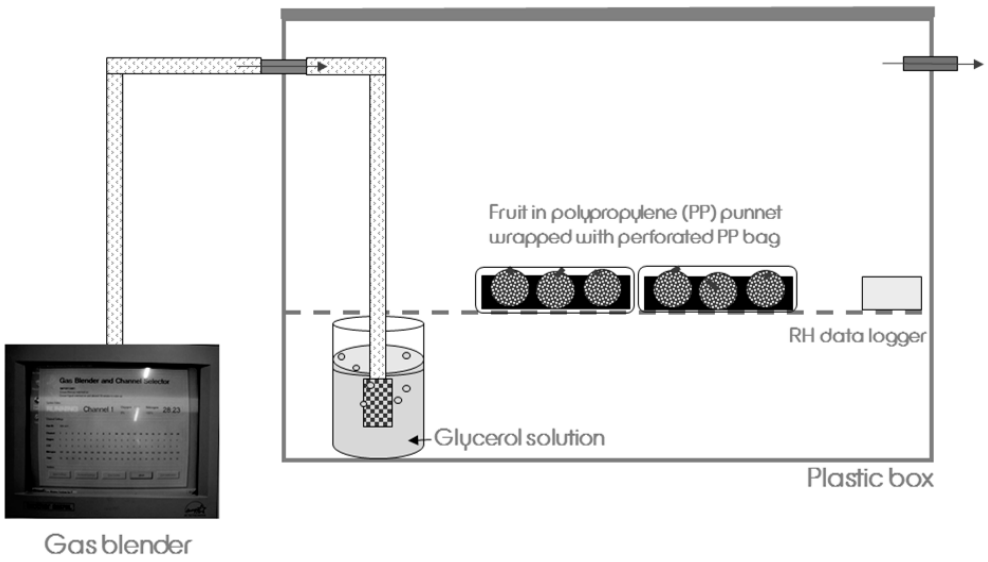


Figure 3.1. Set up for modification of relative humidity in the closed plastic containers using different concentrations of glycerol solution with 3 L min^{-1} flow rate of regular air.

3.1.3. Experiments 6 (Chapter 6)

Litchi cv. Mauritius fruit, grown in Western Galilee, Israel, were exported by Agrexco Agriculture Export Ltd. and imported to the UK (Agrexco, Middx.). Fruit was harvested on 5 July 2008. Fruit was not fumigated with SO_2 and not pre-treated with acid. Litchi fruit were freighted to Agrexco on 7 July 2008 and stored at 1°C before being transported to Cranfield University within 2 h. After pre-cooling at 5°C for 12 h, fruit were sorted for uniformity of size and freedom from defects.

The experiment was arranged as a completely randomised design. Fruit (n = 450) was separated into 5 groups (n = 90), and then divided equally into polypropylene (6 fruits per punnet) plastic punnets: 140 × 115 mm (Nicholas Ltd., Derbys., UK). Four groups of punnetted fruit were then individually placed in either micro-perforated polypropylene (PP) plastic bags of 25 μm thickness (Nicholas Ltd.), PropaFresh™ PFAM (PF), NatureFlex™ NVS (NVS) and Cellophane™ WS (WS) films with 30 μm thickness (Innovia Films Ltd., Cumbria, UK) and sealed using a hand-operated heat sealer (Hulme Martin Ltd.) whilst the last group remained unwrapped (control). Fruit was stored at 13°C (average supply chain temperature; Catto-Smith, 2006) for 9 days and sampled on 0, 2, 4, 6, and 9 days.

3.1.4. Experiment 7 (Chapter 7)

Litchi cv. Mauritius fruit, grown in Western Galilee, Israel, was exported by Agrexco Agriculture Export Ltd. and imported to the UK (Agrexco, Middx.). Fruit was harvested on 6 August 2008. Fruit was fumigated with SO_2 and pre-treated with 19.2 g L^{-1} citric acid solution for 5 min before being air dried otherwise they were free from SO_2 and acid. Litchi fruit was freighted to Luxemburg on 7 August 2008 before being transported by refrigerated-lorry to Agrexco on 8 August 2008. Fruit was stored at 1°C before being transported to Cranfield University within 2 h. After pre-cooling at 5°C for 12 h, fruit were sorted for uniformity of size and freedom from defects.

The experiment was arranged as a completely randomised design. SO_2 free and non-acid treated fruit (non-adulterated fruit; n = 432) and SO_2 and acid treated fruit (commercially-treated fruit; n = 432) were divided equally into PP plastic punnets: 140 ×

115 mm (Nicholas Ltd.). The punneted fruit (n = 6 each punnet) were separated into three groups (commercially-treated n = 144; non-adulterated n = 144 each group). Three groups of commercially-treated or non-adulterated fruit were then individually placed in either PP plastic bags of 25 μm thickness (Nicholas Ltd.) and PropaFresh™ PFAM (PF) (Innovia Films Ltd.) and sealed using a hand-operated heat sealer (Hulme Martin Ltd.) whilst the last group remained unwrapped (control). Fruit were stored at 5 or 13°C (average supply chain temperature) for 11 days and sampled on 0, 3, 7, and 11 days.

3.2. Weight, colour and total soluble solids measurements

Fruits from all experiments were individually weighed and coloured on each outturn (Table 3.1). Colour of fruit pericarp, lightness (L*), colour intensity (C*) and colour (h°; 0° = purple-red and 90° = yellow), were measured using a Konica Minolta colourimeter (Chroma meter model CR-400 and data processor model DP-400, Konica Minolta Sensing, Japan). Each value was the average of three measurement points. Fruit were then peeled, and pericarp, aril tissues and stone separated. Pericarp and aril tissues were weighed. Aril tissue was then gently squeezed for a few drops of juice to determine total soluble solids (TSS) using a digital refractometer (PR 301α, Atago Ltd., Japan). To limit potential contamination of pericarp tissue with aril fruit juice, pericarp tissue was rinsed with deionsied water before snap-freezing. Pericarp and aril tissue were immediately snap-frozen in liquid nitrogen and stored at -40°C before being freeze-dried (Christ LOC-1, Germany) for 5 and 9 days, respectively. Freeze-dry samples were subsequently weighed, ground (aril tissue using hand-operated pestle and mortar, pericarp tissue using a motorized mortar grinder (RMO, Retsch, Germany)) into a fine powder and again stored at -40°C until required.

3.3. Respiration rate, CO_2 and ethylene measurements

Three punnets of fruit (Chapter 4 and 5; n = Table 3.2; 6 and 5 fruit per punnet, respectively) from each treatment were weighed and respiration rate (ml CO_2 kg^{-1} h^{-1}) measured. Fruit (n = Table 3.2) from each punnet were placed into a 450 ml airtight plastic box with a lid fitted with a septum and sealed for 2 h at fruit initial storage temperature

(Chapter 4; 5, 8, 10, 13 or 20°C and Chapter 5; 5 and 13°C). After this incubation period, gas samples were removed with repeated full withdrawal-injection displacements of a 30 ml plastic syringe. The gas sample was immediately analysed for CO_2 using gas chromatography (GC model 8340, DP800 integrator, Carlo Erba Instruments, Herts., UK) coupled to a hot wire detector. The hot wire detector was operated at 120°C and the oven at 80°C. The 2 m long by 4 mm column was packed with 60-80 mesh size Porapak Q (Jones Chromatography, Mid Glamorgan, UK).

For Chapter 6 and 7, CO_2 and ethylene concentrations (n = Table 3.2) from individual PP, PF, WS and NVS (Exp. 6) and PP and PF (Exp. 7) bags were measured by applying a silicone rubber disc to the outer surface (on the top) of the bag and then taking headspace gas samples using a needle and 50 ml syringe. For unwrapped treatment CO_2 in the storage room was analysed. Each gas sample was immediately analysed for CO_2 using gas chromatography (Agilent 6890N Network GC System, USA) coupled with a thermal conductivity detector (TCD) in a Supelco capillary column (30 m long × 530 µm × 0.25 µm film thickness capillary column; Supelco 36245-010A Carboxen 1006 PLOT; Sigma-Aldrich, Dorset, UK). Oven and detector temperatures were set at 200°C with column temperature programmed at 100°C for 2 min and increased to 200°C fo 4 min. Ethylene was analysed with GC8340 gas chromatography with an 980 Flame Ionisation Detector (FID) fitted with Porapak P mesh range 60-80 (Jones Chromatograpphy). The detector and oven were operated at 250 and 100°C, respectively.

The gas chromatography were calibrated with 10.06 kPa CO_2 (10 kPa CO_2, 2 kPa O_2, 88 kPa N_2; Certified Standard from British Oxygen Company, Surrey, UK) whilst ethylene was calibrated against 10.6 $\mu L.L^{-1}$ ethylene balanced in N_2 (BOC).

3.4. Extraction and analysis of sugars

Freeze-dried aril and pericarp powder (150 mg; n = Table 3.2) was extracted with 3 ml of 62.5:37.5 HPLC grade methanol: water (v/v) and mixed well according to Terry *et al*. (2007b). Vials of the slurry were placed in a shaking water bath at 55°C for 15 min. They were removed briefly and shaken for 20 s every 5 min to prevent layering, and then left to cool. The cooled samples were filtered through a 0.2 µm Millex-GV syringe driven filter unit (Millipore Corporation, MA) and the clear extract analysed. The extracts were

stored at -40°C until required. Sugars were measured according to Terry *et al*. (2007b) using a HPLC system comprising a P580 pump and GINA 50 autosampler (Dionex, CA). Extracts were diluted 1:10 (v/v) with HPLC grade water immediately before analysis. The diluted extract (20 µl) was injected into a Rezex RCM monosaccharide Ca^{+} size exclusion column of 300 mm × 7.8 mm diameter, 8 µm particle size (Phenomenex, CA) with a Carbo-Ca^{2+} security guard cartridge of 4 mm × 3 mm diameter (Phenomenex). The mobile phase was degassed HPLC water at a flow rate of 0.6 ml min^{-1}. Column temperature was maintained at 75°C using a Dionex STH column thermostat. Eluted carbohydrates were monitored by an evapourative light scattering detector (ELSD 2420, Waters, MA) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence and abundance of mannose (in pericarp only), fructose, glucose and sucrose were automatically calculated against external standards using Chromeleon version 4.6 software (Dionex, CA). Additionally, mannose was confirmed by an enzyme test kit (K-MANGL 01/05; Megazyme International Ireland Ltd., Co. Wicklow, Republic of Ireland) before being analysed by HPLC.

3.5. Extraction and analysis of non-volatile organic acids

Non-volatile organic acids were extracted according to Terry *et al*. (2007b) with modifications. Freeze-dried litchi aril and pericarp (50 mg; n = Table 3.2) were mixed well with 3 ml of HPLC grade water. The samples were incubated at room temperature for 5 min and filtered through a 0.2 µm filter and stored at -40°C until required. The aril extracts were measured using the same Dionex HPLC as previously described. Samples (20 µl) were injected into an Alltech Prevail Organic Acid column of 250 mm × 4.6 mm diameter, 5 µm particle size (Alltech, IL) with a guard column of 7.5 mm × 4.6 mm diameter. The mobile phase was degassed and filtered 0.2 % (w/v) metaphosphoric acid in HPLC grade water at a flow rate of 1.0 ml min^{-1} (Wang *et al*., 2006). Column temperature was held at 35°C using a Dionex STH column thermostat. Eluted organic acids from aril extracts were monitored using a UVD 170S/340S detector (Dionex). The presence and abundance of oxalic, tartaric, ascorbic, malic and citric acids in aril and pericarp were automatically calculated against external standards using Chromeleon version 4.6 software.

Table 3.2. Number of samples for measurement of weight, sugars, organic acids, anthocyanins, phenols, CO_2 and ethylene in each experiment.

Measurements	Experiments						
	1	2	3	4	5	6	7
Weight: Fruit weight loss, aril and pericarp dry matter and moisture contents	n = 432	n = 324	n = 324	n = 300	n = 300	n = 450	n = 864
Pericarp colour	n = 432	n = 324	n = 324	n = 300	n = 300	n = 450	n = 864
Total soluble solids	n = 432	n = 324	n = 324	n = 300	n = 300	n = 450	n = 864
Sugars: aril	n = 432	n = 324	n = 324	n = 300	n = 300	n = 225	n = 288
Sugars: pericarp	n = 432	n = 324	n = 324	n = 300	n = 300	n = 225	n = 288
Organic acids: aril	n = 432	n = 324	n = 324	n = 300	n = 300	n = 225	n = 288
Organic acids: pericarp	n = 432	n = 324	n = 324	n = 300	n = 300	n = 225	n = 288
Anthocyanins	n = 432	n = 324	n = 324	n = 300	n = 300	n = 225	n = 288
Total phenols: aril	n = 432	n = 324	n = 324	-	-	-	-
Total phenols: pericarp	n = 432	n = 324	n = 324	-	-	-	-
Respiration rate	n = 72	n = 54	n = 54	n = 150	n = 150	-	-
CO_2	-	-	-	-	-	n = 90	n = 72
Ethylene	-	-	-	-	-	n = 90	n = 72

3.6. Extraction and analysis of anthocyanins

Pericarp tissue was extracted and quantified according to Terry *et al.* (2007b) and Giné Bordonaba and Terry (2008) with modifications. Freeze-dried litchi pericarp powder (150 mg; n = Table 3.2) was mixed well with 3 ml of 70:29.5:0.5 HPLC grade methanol:water:HCl (v/v/v). The samples were held at 35°C for 1.5 h. They were shaken for 10 s every 15 min to prevent layering. The samples were filtered as before and stored at -40°C until required. Pericarp extract (20 µl) was quantified using an Agilent 1200 series HPLC (Agilent, Berks., UK) and injected into a Zorbax column of 250 mm × 4.6 mm diameter, 5 µm particle size with 4 XDB-C18 (5 µm) guard column of 12.5 mm × 4.6

mm diameter (Agilent). The mobile phase was degassed and filtered (A) 1 % (v/v) phosphoric acid and 10 % (v/v) acetic acid in water and (B) acetonitrile with a flow rate of 1 ml min^{-1}. The program followed a linear gradient from 2 to 20 % of B in 25 min and then from 20 to 40 % of B in 15 min. Anthocyanins were detected using a photodiode array detector (G1315D, Agilent) at 520 nm. Column temperature was set at 40°C and the temperature of the autosampler held at 4°C. The presence and abundance of cyanidin 3-glucoside, cyanidin 3-rutinoside and malvidin 3-glucoside were automatically calculated against external standards (Extrasynthese, Lyon, France) using Chemstation Rev. B.02.01 software (Agilent).

3.7. Extraction and analysis of total phenolic compound

Total phenolics were extracted and measured according to the Folin–Ciocalteu Method (Thanaraj *et al.*, 2009) with slight modifications based on the reduction of a phosphowolframate–phosphomolybdate complex by phenolics to blue reaction products. Briefly, either freeze-dried litchi aril or pericarp powder (150 mg; Table 3.2) were dissolved in 3 mL of 80 ethanol: 20 water (v/v) and held in a water bath for 2 h at 70°C, mixing every 20 min. The solution obtained was filtered as before and the clear filtrate analysed. Twenty microlitres of filtrate and 3.2 ml of distilled water were mixed with 200 µL of Folin–Ciocalteu's phenol reagent, followed by 600 µL of sodium carbonate (1.9 M). After 2 h incubation at room temperature (20°C) in the dark, absorbance was measured at 765 nm using a Camspec M501 UV/VIS spectrophotometer (Camspec Ltd., Cambridge, UK). Phenol content was estimated from a standard curve of gallic acid and results expressed as mg gallic acid equivalents (GAE) g^{-1} dry weight (DW).

3.8. Fruit decay analysis

Disease was recorded by scoring in percentage of incidence on each fruit surface. Disease was scored using a 1-5 visual scale (1 = no incidence, 2 = one spot to 5% of disease on each fruit surface, 3 = 10% on fruit surface, 4 = 15% on fruit surface, 5 = 20% on fruit surface).

3.9. Statistical analyses

All statistical analyses were carried out using Genstat for Windows Version 10.1 (VSN International Ltd., Herts., UK). Analysis of variance was performed on the data, extracting information about the main effects and interactions of storage RH, temperature and storage duration. Least significant difference values (LSD; $P < 0.05$) were calculated for comparison of appropriate treatment means. Unless otherwise stated significant differences were $P < 0.001$. Principal component analysis (PCA) (using group average linkage) was carried out on the auto-scale data set of each cultivar using Unscrambler® Camo Software AS version 9.8 (free trial; www.camo.com), in order to understand the effect of temperature and RH level on chemometric profile of spatial and temporal variation within each cultivar.

CHAPTER FOUR

Influence of different storage temperatures on physiological and biochemical profiles of both aril and pericarp tissue in imported litchi fruit

4.1. Abstract

Optimum storage temperature during distribution not only controls decay but also reduces the metabolic processes in harvested fruit. However, previous studies have mainly considered the influence of temperature on litchi fruit pericarp discolouration. The aim of this chapter, thus, was to determine the physiological and biochemical alterations in litchi fruit during storage at different temperatures. The physiological and biochemical changes of litchi fruit cv. Mauritius stored at 5, 8, 10, 13, and 20°C for 13 days were studied in 3 experiments. Respiration rate of fruit kept at 5, 8 and 10°C generally declined during 13 days but increased at 13°C from day 7 and increased rapidly at 20°C from day 3. Weight loss of fruit slightly increased at all temperatures, and markedly increased at 20°C. Pericarp colour, assessed using lightness (L*), chroma (C*), and hue (h°), decreased at all temperatures, with the greatest decrease occurring in fruit stored at 20°C. Total soluble solids (TSS) declined at all storage temperatures but was maximal at either 13 (Exp. 1) or 20°C (Exp. 2 and 3). Sucrose from aril tissue decreased at all temperatures whilst glucose and fructose increased over 13 days. Patterns of changes in concentrations of ascorbic, citric, malic, oxalic and tartaric acids from aril tissue were not consistent, but all acids in pericarp tissue at all temperatures declined during 13 days with the smallest changes in fruit stored at 5°C. Total phenolics, cyanidin 3-glucoside, cyanidin 3-rutinoside and malvidin 3-glucoside concentration in pericarp tissue of fruit from all temperatures generally decreased during 13 days but were slightly higher at 5°C. The total phenolic content of aril tissue declined at all temperatures, and contained lower concentrations than pericarp tissue. In conclusion, whilst the optimum temperature for long-term storage is 5°C, postharvest eating quality could be adequately maintained at 8 or 10°C.

4.2. Introduction

Refrigerated storage is one of the most effective methods used in extending the postharvest life of fresh horticultural commodities. Low temperature can reduce the fruit metabolism and can also affect the rate of growth and spread of pathogens and decay. For instance, cold storage can delay postharvest deterioration in rambutan and longan fruit (Jaing *et al*., 2002; Kondo *et al*., 2005). The effects of storage temperature on some litchi cultivars are shown in Table 4.1. Although low temperatures have been reported to prolong postharvest life of litchi fruit, the detailed effects of temperature on physiological and chemical changes during storage have not been fully defined. Thus, the aim of this study was to detail the specific spatial and temporal physiological and biochemical changes in litchi fruit as affected by different storage temperatures, with specific emphasis on non-structural carbohydrates, non-volatile organic acids and phenylpropanoids in both aril and pericarp tissue. The results from Chapter 4 were then used to inform the design of the following experiments, which were planned to study the alterations in physiology and biochemistry of imported litchi fruit stored under different vapour pressure deficits (Chapter 5).

Table 4.1. Effects of storage temperature on harvested litchi fruit.

Cultivars	Storage temperature (°C)	Storage Time (days)	Results (Changes between beginning and end of storage time)					References
			Browning (% at end of storage)	TSS	Titratable acidity	Weight Loss	Other	
McLean's Red	2.0	34	20	n/a	n/a	↑	Sensory: accepted	Sivakumar and Korsten, 2006
Bombay	2.0	56	n/a	↑	↓	↑	Firmness ↑	Mahajan and Goswami, 2004
Huaizhi	4.0	35	n/a	n/a	n/a	n/a	Phenolics ↓	Zhang *et al*., 2000
Wai Chee	5.0	14	60-84	n/a	↓	n/a	Disease ↓	Jacobi *et al*., 1993
Hong Huay	5.5	15	20	↓	↓	n/a	-	Ketsa and Leelawatana, 1992
Seedless Late	0.0-3.0	19	n/a	n/a	n/a	↑	-	Sandhu and Randhawa, 1992
Hei Ye	5.0	27	n/a	↓	↓	n/a	-	Huang and Wang, 1990
Hei Ye	2.0	30	90	↓	↓	↑	Res[1]., Anth[2].,SC[3].↓	Paull and Chen, 1987
Haak Yip	7.0	40	46	↓	↓	↑	CI[4] (<7C,>30days)	Tongdee, *et al*., 1982

[1] Respiration, [2] Anthocyanins, [3] Sucrose, [4] Chilling injury

Arrow indication: ↑ Increase, ↓ Decrease

n/a: not applicable; TSS: total soluble solids

4.3. Materials and methods

Sample preparation for Chapter 4 was described in section 3.1.1. The measurement and analysis of weight loss, moisture content, respiration rate, TSS, pericarp colour, individual sugar, individual organic acid, individual anthocyanin and total phenolic compound were described in Chapter 3: Methodology.

4.4. Results and discussion

4.4.1. Fruit weight loss, dry matter and moisture content

It has previously been reported that higher storage temperature resulted in higher weight loss in litchi fruit (Tongdee *et al*., 1982; Underhill and Critchley, 1994; Jiang and Fu, 1999). Weight loss of fruit from Exp.2 and 3 kept at 20°C were 6.09 and 6.87-fold higher than those stored at 5°C, whilst storage at 13°C resulted in 1.42, 1.11 and 1.34-fold (Exp. 1, 2 and 3, respectively) higher weight loss than fruit stored at 5°C (Figure 4.1). Higher temperature causes water molecules to have more free energy, which can accelerate their movement which is important for exchange to the atmosphere around the produce (Kays and Paull, 2004) resulting in faster evapouration. Higher temperature requires more moisture to saturate the air. A greater difference in vapour pressure between the produce and the storage atmosphere can lead to faster moisture loss from the fruit to the environment (Will *et al*., 1981; Paull, 1999). In the current study, the air at 20°C would have contained more moisture vapour than the air at 5, 8, 10 and 13°C. The drier air at the higher temperatures resulted in more rapid fruit weight loss. The results indicated that higher moisture loss was induced by higher temperature, possibly together with low relative humidity (see section 5.4.1, Chapter 5).

The pericarp moisture content of litchi fruit stored (in Exp.1-3) at 5°C were higher than those kept at other storage temperatures (Figure 4.2) whereas there was no significant difference between aril moisture content in all experiments. The results indicated that fruit weight loss is mainly caused by pericarp dehydration which was consistent with studies in litchi fruit cv. Kwai Mi reported by Joas *et al*. (2005).

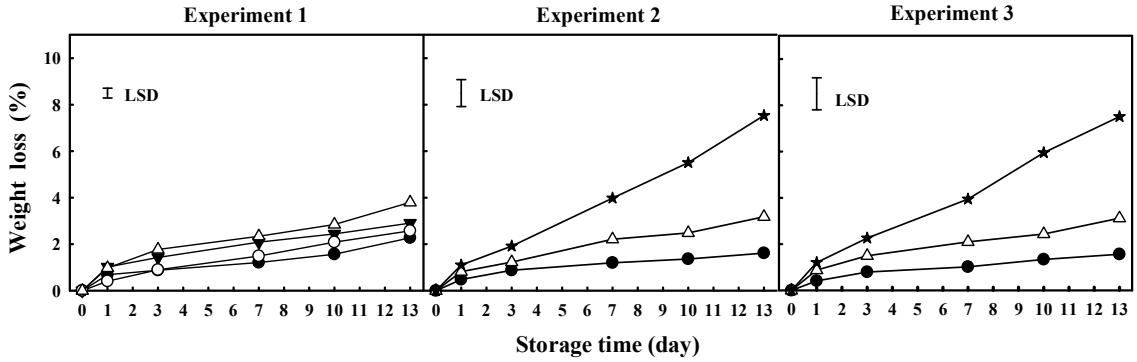


Figure 4.1. Weight loss (%) of litchi cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, Symbols correspond to temperature; n =18.

4.4.2. Respiration rate

In all three experiments the respiration rate was proportional to temperature with the lowest respiration rate at the lowest temperature (Figure 4.3). Similar results were shown for cv. Haak Yip by Tongdee *et al*., (1982). A decline in respiration rate was recorded in fruit from all temperatures over storage time. A slight increase in respiration rate for Exp. 1, 2 and 3 at 13°C was detected after day 7. Whilst the respiration rate of fruit held at 20°C in both Exp. 2 and 3 considerably increased after 1 day of storage. Although the increased respiration rate of fruit at 13 and 20°C could be due partly to pathogenic infection, it is more likely to have been affected by pericarp desiccation and browning (Chen *et al*., 1987; Zhang and Quantick, 2000). Pericarp lightness (L*) in Exp. 1, 2 and 3 was correlated with pericarp moisture content (r = 0.761, 0.686 and 0.658, respectively). The results indicated that lower storage temperatures minimised fruit dehydration and moisture loss by maintaining moisture vapour in the air surrounding the fruit leading to a lower respiration rate over the time.

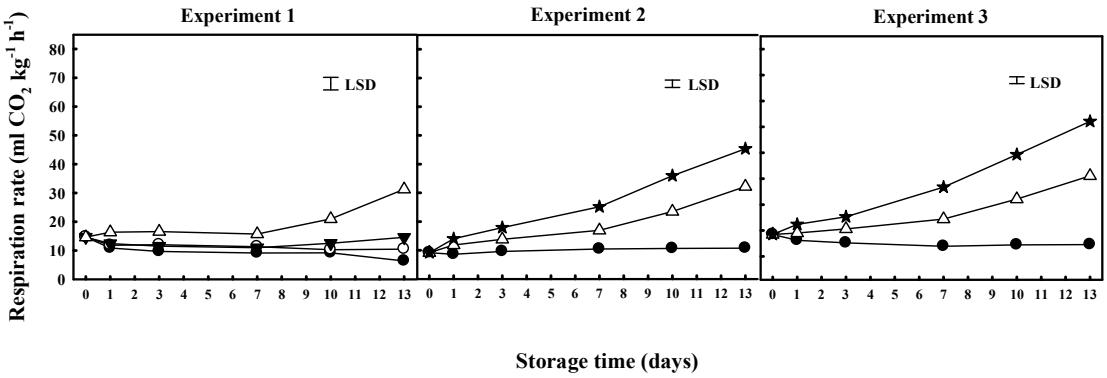


Figure 4.2. Respiration rate of litchi cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, Symbols correspond to temperature; n = 3.

4.4.3. Pericarp colour

Pericarp colour (L*, C*, h°) from all storage conditions significantly decreased during storage time, with the colour of fruit kept at 5°C in all three experiments declining most slowly (Figure 4.4). Fruit kept at 13 (Exp. 1-3) and 20°C (Exp. 2-3) had faster decreases in L* (darker), C* (less colour intensity) and h° (darker red) values than those at the other temperatures. However, there was no significant difference in pericarp colour between fruit kept at 5, 8 and 10°C in Exp. 1. The results indicated that litchi pericarp browning was reduced when fruit were stored at 5, 8 and 10°C compared to the other temperatures. The lower respiration rates and water loss in fruit stored at 5, 8 and 10°C could have reduced the desiccation and browning reaction rate (Huang *et al*., 1990; Jacobi *et al*., 1993; Zhang and Quantick, 2000; Mahajan and Goswami, 2004) and hence led to a brighter red coloured pericarp compared to those fruit kept at 13 or 20°C. The h° values recorded in the present study declined during 13 days storage which was in disagreement with the studies of Huang *et al*. (1990; cultivar not mentioned, at 4°C for 29 days), Chaiprasart (2005; cv. Hong Huay, at 5°C for 12 days) and Archibald and Bower (2008; cultivar not mentioned, at 1 and 5.5°C for 40 days) who reported no change in h°. The alteration in pericarp colour, however, might be due to differences in fruit maturity, storage duration and acid treatment. In this study, pericarp colour developed from colour break stage (h° = 53.34) to red colour (h° = 39.30) after 13 days storage.

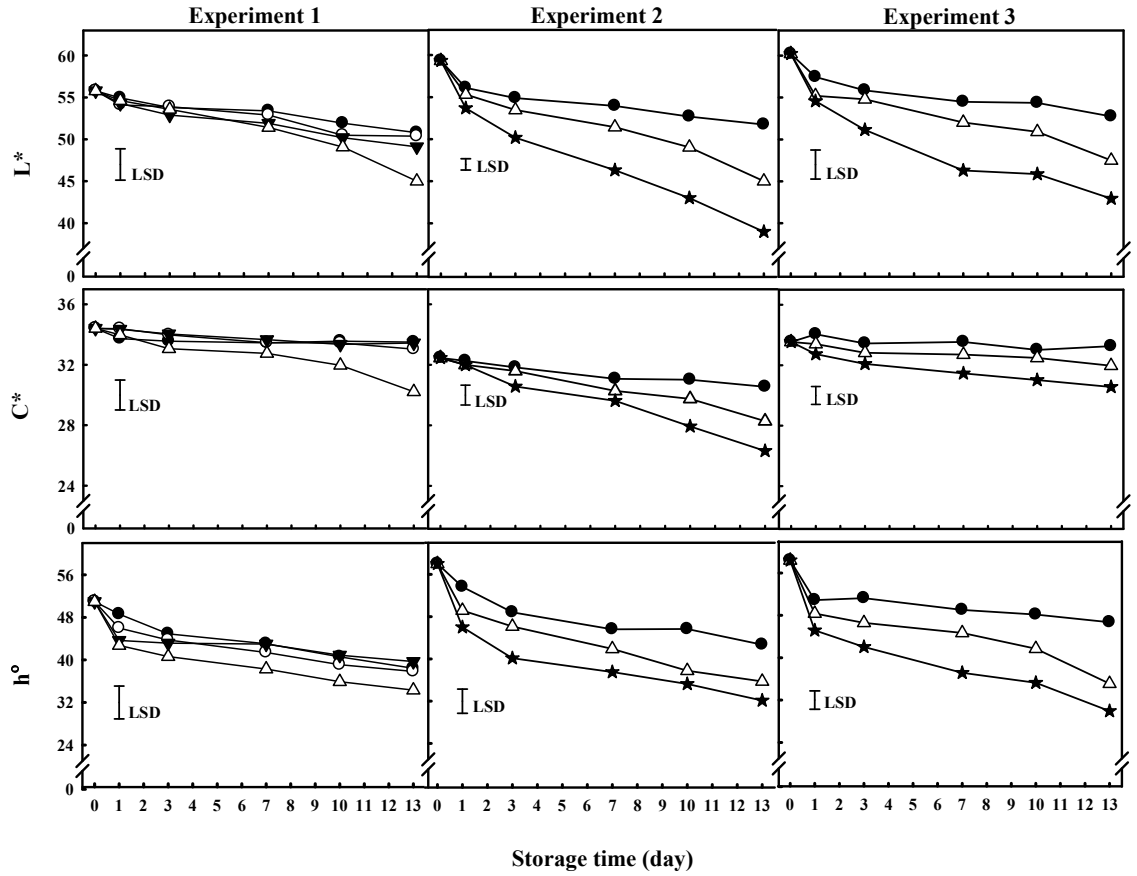


Figure 4.3. Pericarp colour (L*, C* and h°) of litchi cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, Symbols correspond to temperature; n =18.

4.4.4. Total soluble solids and sugars concentration

In Exp. 1, 2 and 3, total soluble solids (TSS) contents in fruit stored at 5°C (17.82, 18.12 and 17.59 %, respectively) were significantly higher than in those stored at 13 or 20°C and declined over storage time (Figure 4.4). Litchi aril from Exp. 1, 2 and 3 contained mainly glucose (275.5, 336.1 and 382.9 mg g^{-1} DW, respectively), fructose (270.5, 331.9 and 347.6 mg g-^{1} DW, respectively) and sucrose (170.2, 64.2 and 37.68 mg g^{-1} DW, respectively). Glucose and fructose content of fruit from all treatments increased during storage whilst sucrose decreased. This is probably due to hydrolysis of sucrose to fructose and glucose, and sugar invertase activity (Chan *et al*., 1975). Fruit stored at 5°C had significantly higher sucrose and lower glucose and fructose than those stored at 8, 10, 13 or 20°C (Figure 4.5). Total sugars (glucose + fructose + sucrose) and calculated sweetness (from the following equation: 0.6 × glucose + 1.8 × fructose + 1.0 × sucrose, Keutgen and Pawelzik, 2008) decreased over time in all experiments but was not affected by storage temperature. Paull and Chen (1987) reported that decrease of sucrose content correlated well with reduction of TSS (from 18.5 to 15.5%, cvs. Hei Ye and Chen Yi, kept at 22°C for 8 days in polyethylene bag). However, there was no correlation between TSS content and sucrose, glucose, fructose, total sugar or calculated sweetness (Figure 4.6A, B and C) in the current study. The results could indicate that TSS is in fact not a suitable predictor of litchi sugar content. The unsuitability of TSS as sweetness indicator in these experiments could be explained by the relatively small changes in the refractometer measurements in the litchi fruit (from 18.5 to 16.5%). This is confirmed by the slight changes of TSS found in litchi cvs. Da Zao, Hei Ye, Bengal, Huai Zhi (Batten, 1989) and Brewster (Finger *et al*., 1997).

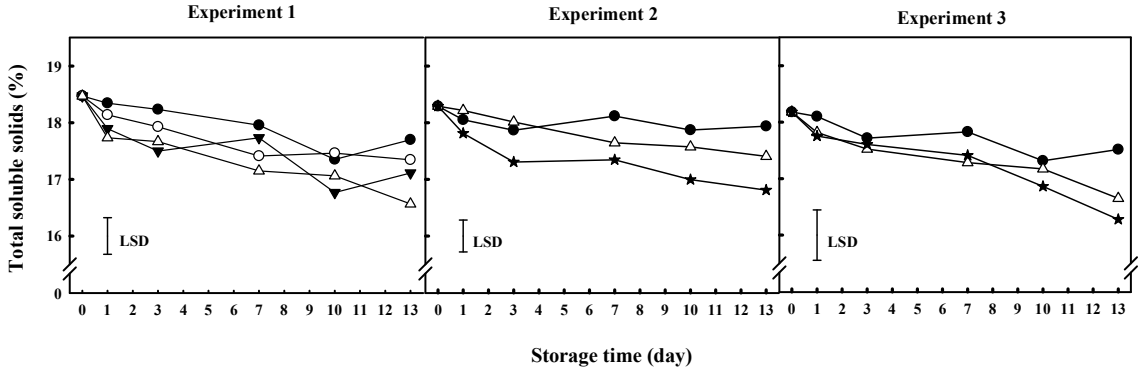


Figure 4.4. Total soluble solids (TSS; %) of litchi cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, Symbols correspond to temperature; n =18.

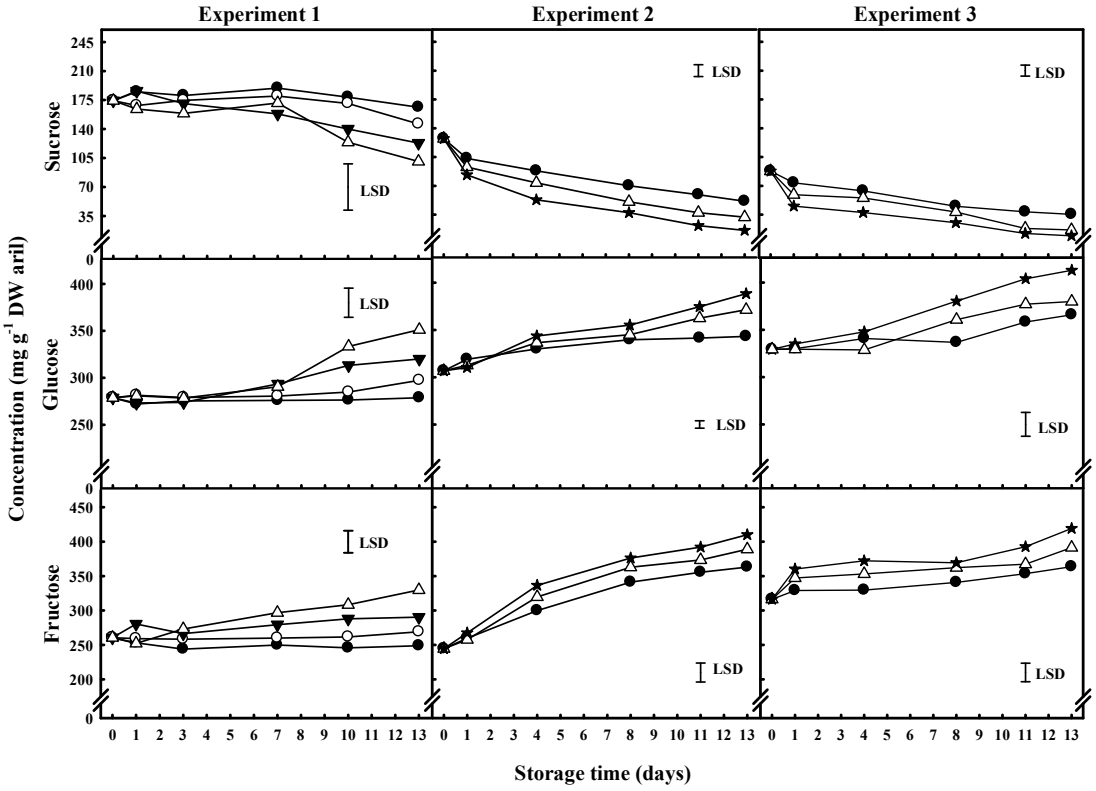


Figure 4.5. Sucrose, glucose and fructose concentration in dry litchi aril cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, symbols correspond to temperature; n =18.

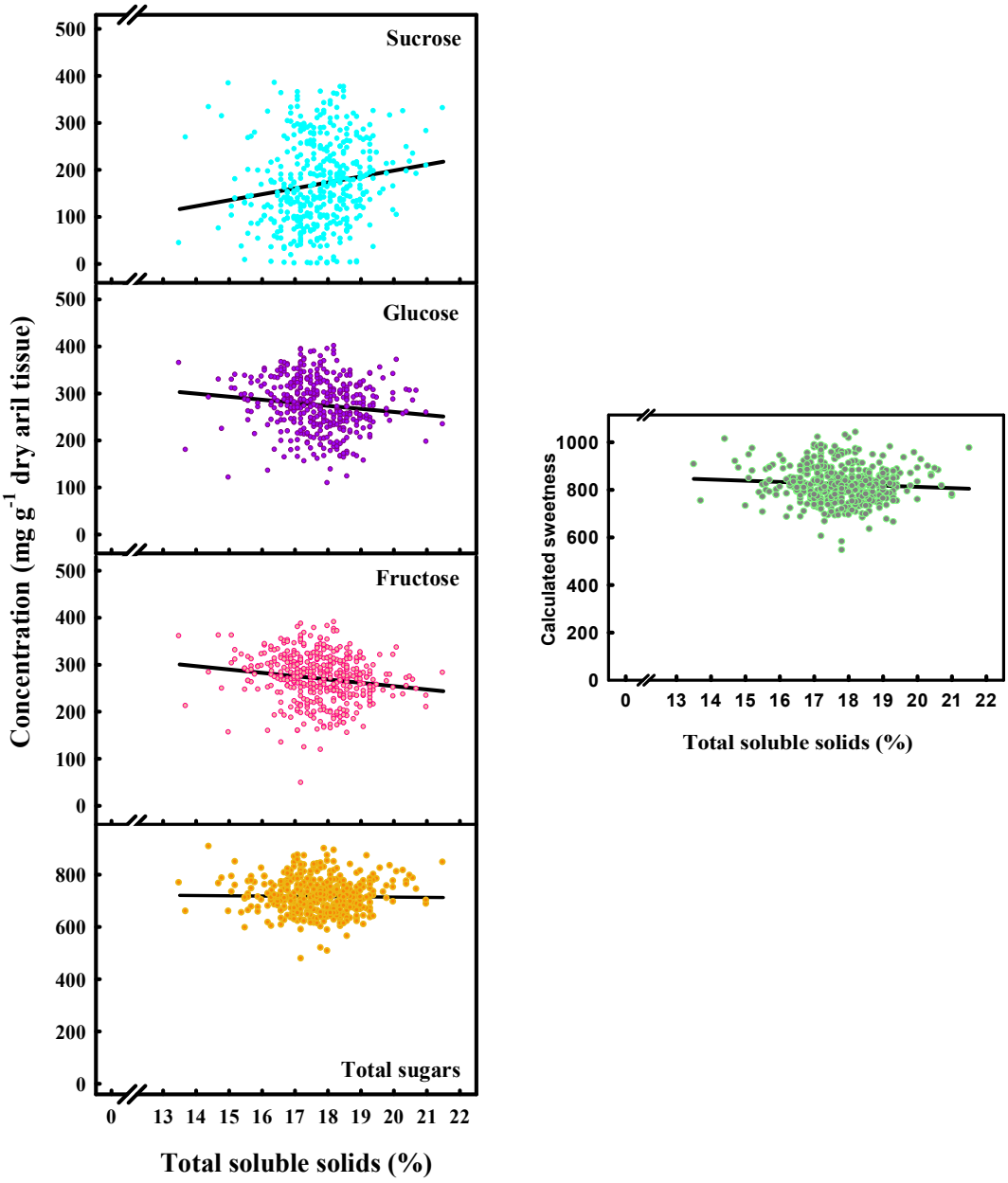


Figure 4.6A. Correlations between total soluble solids and sucrose, glucose, fructose, total sugars and calculated sweetness in litchi aril in Exp. 1 (n = 432).

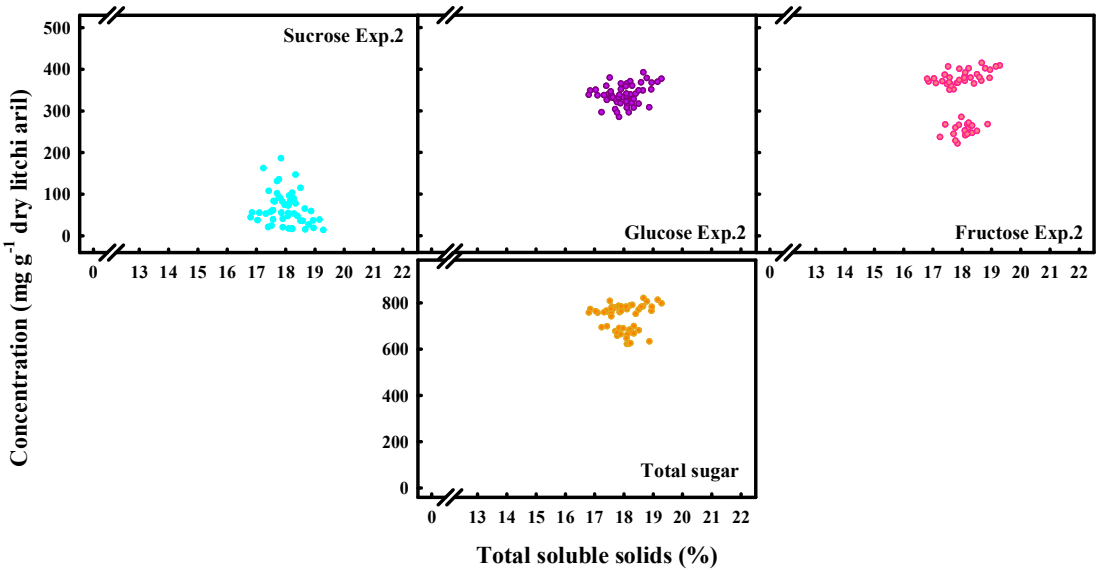


Figure 4.6B. Correlations between total soluble solids and sucrose, glucose, fructose and total sugars in litchi aril in Exp. 2 (n = 54)

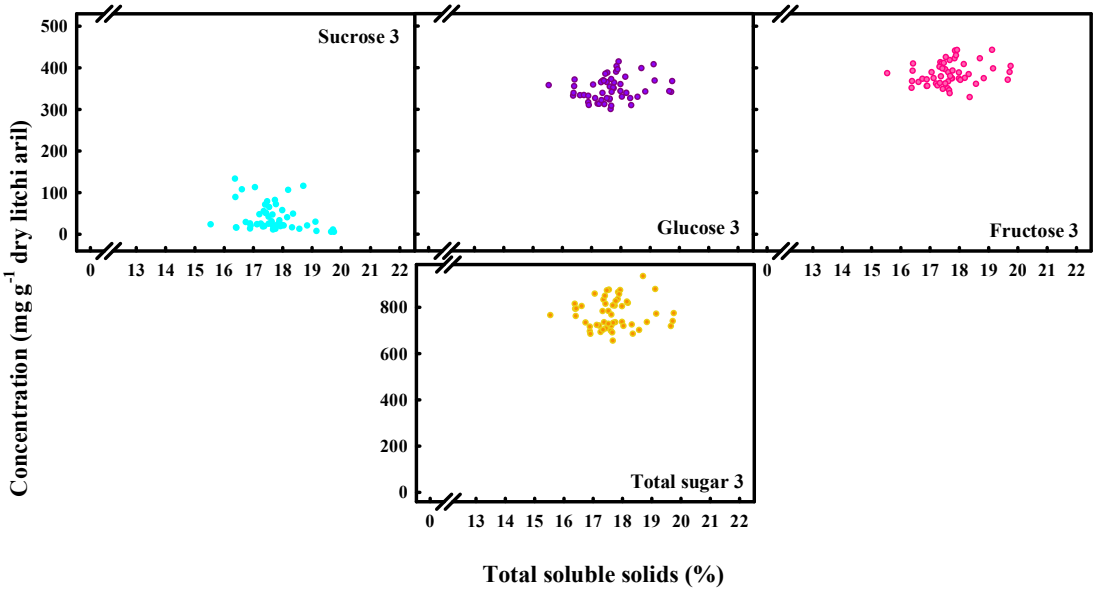


Figure 4.6C. Correlations between total soluble solids and sucrose, glucose, fructose and total sugars in litchi aril in Exp. 3 (n = 54).

4.4.5. Organic acids concentration

The organic acids found in litchi aril were ascorbic, citric, malic, oxalic and tartaric acids (Figure 4.7.). The aril acid present in the greatest concentration was malic acid (Exp. 1: 22.66, Exp. 2: 22.20, Exp. 3: 14.86 mg g^{-1} DW), followed by tartaric, ascorbic, citric, and oxalic acids, respectively. Fruit stored at 5°C had the greatest reduction in ascorbic, citric, oxalic, and tartaric acids followed by 8, 10, 13 and 20°C. The concentrations of malic, ciric and ascorbic acids in the three experiments generally declined after 13 days storage. Organic acids in the arils are one of major carbon sources for the respiratory tricarboxylic acid cycle and other general metabolic processes in stored fruit (Siriphanich, 2006) and this could lead to the decline in aril organic acids. Decreases in acid concentration during storage were recorded in stored litchi fruit cvs. Kom (see section 5.4.5, Chapter 5) and Bombay (Mahajan and Goswami, 2004). Tongdee *et al*. (1982) and Huang and Xu (1983) found that the degree of sweetness of matured litchi was due mainly to a decrease in acidity. However, acidity alone might not adequately explain litchi taste. The sugar:acid ratio has been recommended as an alternative indicator (Batten, 1989) to ensure the taste in harvested litchi fruit. The sugar:acid ratio in the present study was higher in fruit stored at 20 (32.55:1) or 13°C (25.42:1) than at 5°C (15.99:1), and is relatively high compared to the ratio in litchi cvs. Purbi (8.05:1) and Bedana (6.40:1) (titratable acidity (TA) and total sugar measurements based on AOAC method (1984); Waseem *et al*., 2002). These differences are probably due to variations in cultivar, growing location, climate and stage of maturity at harvest or perhaps the extraction procedure employed. Sugar:acid ratio was negatively correlated with malic acid (Figure 4.8) and total acid concentration (r = -0.822). Organic acids, particularly malic acid, might therefore be a key taste-related compound in litchi arils. Results indicated that storage at 5°C maintained better taste-related compounds in litchi fruit compared to those stored at 13 and 20°C.

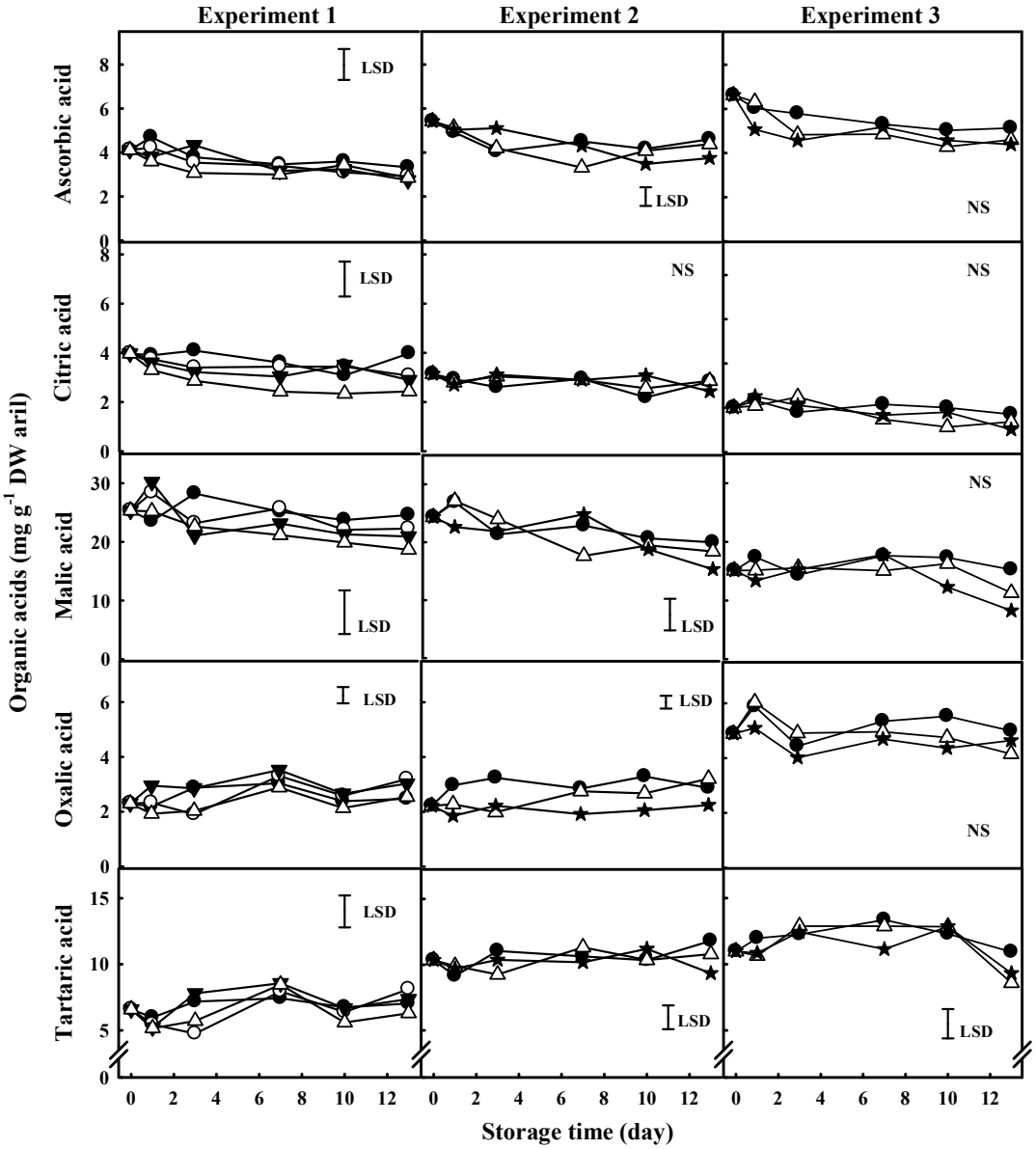


Figure 4.7. Ascorbic, citric, malic, oxalic and tartaric acid concentration in dry litchi aril cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, Symbols correspond to temperature; n =18.

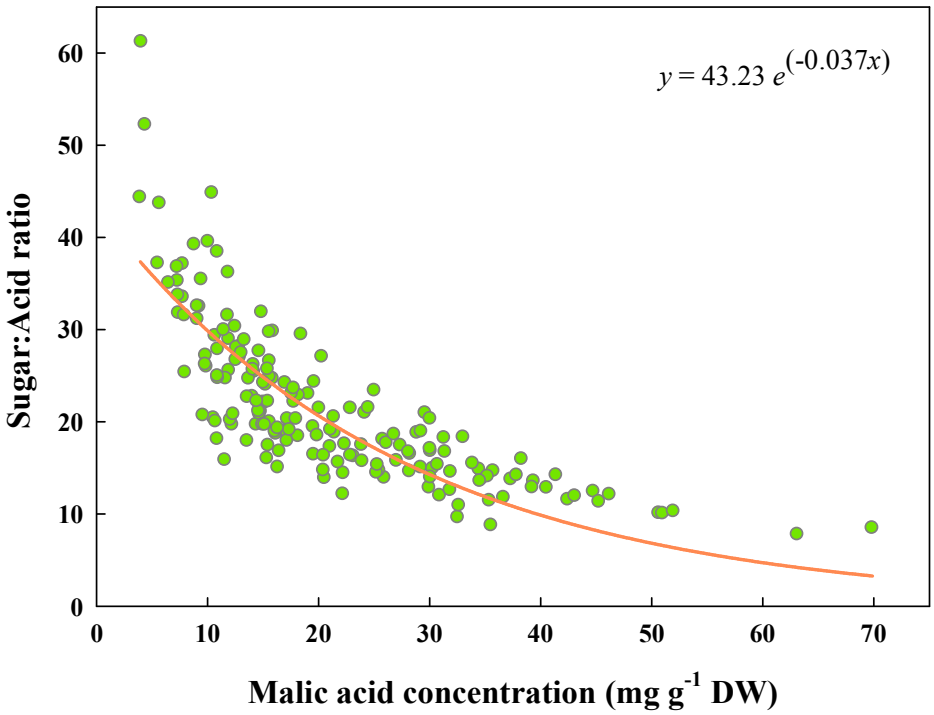


Figure 4.8. Correlation between malic acid and sugar:acid ratio in litchi cv. Mauritius aril after 13 days at 5 or 13°C storage from Exp. 1 (n = 216; *r* = -0.822; y = sugar:acid ratio; x = malic acid concentration).

Malic acid was found in the highest concentration in the pericarp tissue (Exp. 1: 3.98, Exp. 2: 7.05, Exp. 3: 8.23 mg g^{-1} DW), followed by tartaric (Exp. 1: 4.17, Exp. 2: 6.21, Exp. 3: 7.31 mg g^{-1} DW) and citric acids (Exp. 1: 3.49, Exp. 2: 4.68, Exp. 3: 4.11 mg g^{-1} DW), and with small amounts of ascorbic and oxalic acids (Figure 4.9). As in aril tissue, organic acids in pericarp tissue decreased during storage but was significantly higher in fruit stored at 5°C, followed by those stored at 8, 10, 13 and 20°C. Decreases in organic acids can be beneficial in terms of the antioxidant properties of pericarp tissue by enhancing membrane integrity and retaining peroxidise (POD) and polyphenol oxidase (PPO) activities which can lead to a reduction in phenolic and anthocyanin concentrations (Joas *et al*., 2005; Zheng and Tian, 2006).

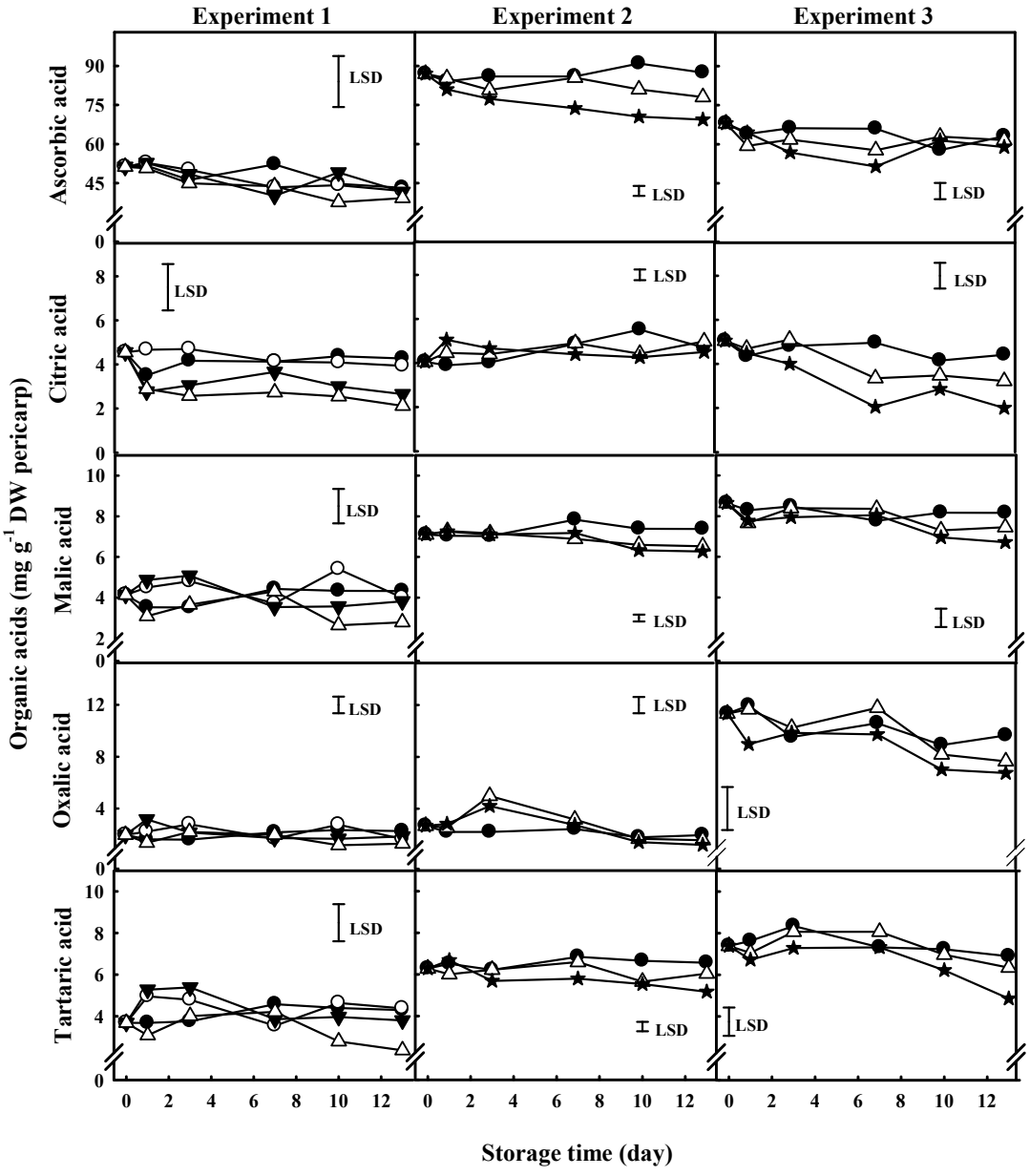


Figure 4.9. Ascorbic, citric, malic, oxalic, tartaric acid concentration in dry litchi pericarp cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown and symbols correspond to temperature; n =18.

4.4.6. Anthocyanins concentration

The anthocyanins in litchi aril were cyanidin 3-glucoside, cyanidin 3-rutinoside and malvidin 3-glucoside. Cyanidin 3-rutinoside was present in the greatest concentration (Exp. 1: 596, Exp. 2: 902, Exp. 3: 1066 μg g^{-1} DW) followed by cyanidin 3-glucoside (Exp. 1: 55.1, Exp. 2: 63.0, Exp. 3: 73.6 μg g^{-1} DW), and with lowest contents of malvidin 3-glucoside (Exp. 1: 6.34,Exp. 2: 9.05, Exp. 3: 13.13 μg g^{-1} DW). All anthocyanins in all three experiments generally declined during 13 days storage. However, fruit stored at 5°C had the highest concentration of cyanidin 3-rutinoside (Exp. 1: 564; Exp. 2: 1000; Exp. 3: 1038 μg g^{-1} DW), cyanidin 3-glucoside (Exp. 1: 46.8; Exp. 2: 69.0; Exp. 3: 86.7 μg g^{-1} DW) and malvidin 3-glucoside (Exp. 1: 5.11; Exp. 2: 9.03; Exp. 3: 15.21 μg g^{-1} DW) followed by fruit kept at 8, 10, 13 and 20°C, respectively (Figure 4.10). The reduction in anthocyanin concentrations over storage time could be mainly caused by an increase in the enzymatic activities of PPO, POD and anthocyanase (Huang *et al*., 1990; Jiang *et al*., 2006) in pericarp tissue which possibly resulted in pericarp browning. However, there was no correlation between pericarp discolouration and anthocyanin degradation in these three experiments. Underhill and Critchley (1994) reported that the red colour in litchi fruit was better correlated with pH level in pericarp tissue than with the concentration of anthocyanins. A rise in pH or a decrease in acid concentration was shown to convert the red flavylium cations into a colourless form, which allows the brown colour to appear (Gross, 1987; Holcloft and Micham, 1996). Therefore, decreases in the concentration of acids in the litchi pericarp were probably associated with anthocyanin degradation and increases in pericarp browning.

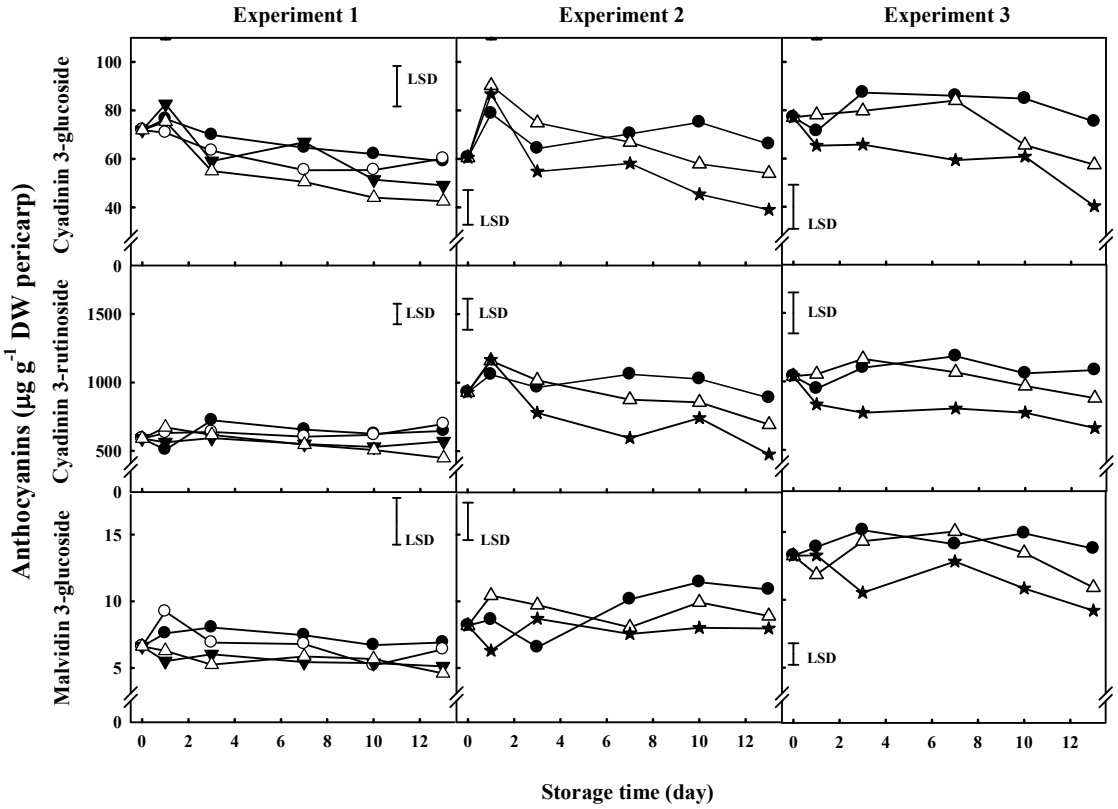


Figure 4.10. Cyanidin 3-glucoside, cyanidin 3-rutinoside and malvidin 3-glucoside concentration in dry litchi pericarp cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, symbols correspond to temperature; n =18.

4.4.7. Total phenolic concentration

Total phenolic concentrations in aril tissue in Exp. 1, 2 and 3 were 1.862, 2.036, and 2.337 mg GAE g^{-1} DW, respectively and generally declined during 13 days storage. Fruit stored at 5°C had the highest total phenolic concentration (Exp. 1: 2.187; Exp. 2: 2.004; Exp. 3: 2.342 mg GAE g^{-1} DW) over storage time, followed by fruit stored at 8, 10, 13 and 20°C, respectively (Figure 4.11). The lower respiration rate and fruit weight loss in fruit kept at 5°C could slow cell decompartmentalisation in aril tissue resulting in a reduction in the interactions between phenolic substrates and PPO and POD (Jiang, 2000). A decrease in aril phenolic content was previously recorded in litchi fruit cvs. Rose (Shah and Nath, 2008) and Feizixiao (Wu *et al*., 2001) stored at 4°C for 4 and 13 days, respectively.

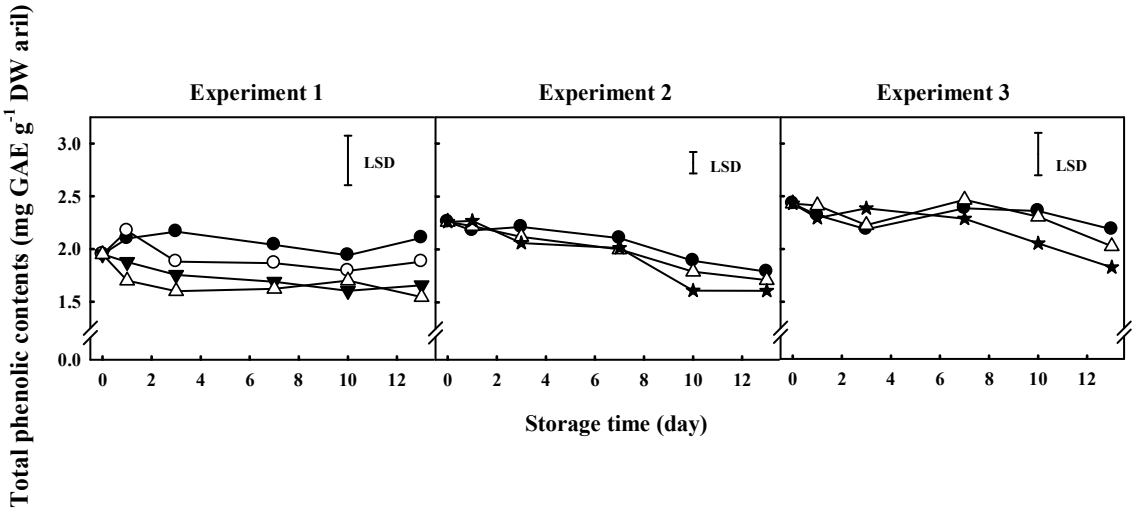


Figure 4.11. Total phenolic concentration in dry litchi aril cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, symbols correspond to temperature; n =18.

Total phenolic compounds in the pericarp tissue was greater than in aril tissue, and were 80.2, 61.66, and 64.3 mg GAE g^{-1} DW in Exp. 1, 2 and 3, respectively. The content of total phenolic compounds in the pericarp tissue varied significantly with temperature. Fruit stored at 5°C had the highest concentration of total phenolics (Exp. 1: 86.5; Exp. 2:

87.09; Exp. 3: 69.00 mg GAE g^{-1} DW), followed by those stored at 8, 10, 13 and 20°C. Total phenolic contents in pericarp tissue decreased over time in all three experiments, corresponding with the changes observed in aril tissue. However, total phenolics in Exp. 2 increased between days 0 and 3 at all storage temperatures. According to Zhang and Quantick (2000), litchi fruit is normally harvested at approximately 80% maturation and at this maturity, fruit cells are still able to maintain the integrity of the membrane system which controls the interactions between phenolic compounds and enzymes. However, it was reported that longer storage periods led to enhancement of cell decompartmentalisation, PPO and POD activities, and phenylalanine ammonialyase (PAL) content (Zhang and Quantick, 2000; Jiang, 2000; Jiang *et al*., 2004; Jiang *et al*., 2006). These processes were found to promote decreases in phenolics in litchi pericarp during storage. Also, PAL induction can be influenced by light, plant hormones, wounding and disease (Kays and Paull, 2004; Siiphanich, 2006). Disease detected in fruit stored at 13 or 20°C probably accelerated the production and activity of PAL resulting in higher losses of phenolic compounds during storage.

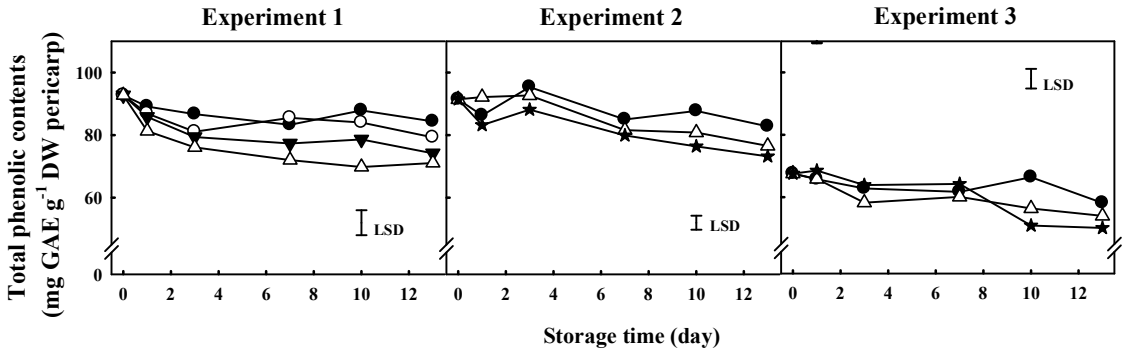


Figure 4.12. Total phenolic concentration in dry litchi pericarp cv. Mauritius during 13 days storage at 5 (●), 8 (o), 10 (▼), 13 (Δ) and 20°C (∗). LSD (P = 0.05) bars are shown, symbols correspond to temperature; n =18.

4.4.8. Fruit decay

Pathogenic infections were observed in fruit stored at 13 and 20°C whilst no disease was found in fruit kept at 5°C in all experiments and at 8 or 10°C in experiment 1 (Table 4.2; Appendix B). *Aspergillus*, *Penicillium* and *Rhizopus* are the major postharvest pathogens found of litchi fruit (Prasad and Bilgramim, 1973; Jiang *et al*., 2003; Appendix C). Production of CO_2 from microbial respiration could result in a higher CO_2 concentration in the fruit chambers and result in the increased respiration rate recorded in fruit stored at 13 and 20°C after 3 and 1 days storage, respectively (Figure 4.2). Although greater respiration rate of fruit kept at 13 and 20°C was partially affected by pathogens, the storage temperature (either 13 or 20°C) was the principal factor to enhance the respiration rate in stored fruit over time. According to Figure 4.2, the respiration rate of fruit treated at 5°C (all experiments) was significantly lower than those fruit stored at 13 (Exp.1-3) and 20°C (Exp.2-3). An increase in respiration rate in stored litchi fruit was also reported by Zhang and Quantick (2000; cv. Huaizhi at 4°C for 35 days) and Pesis *et al*. (2002; cv Mauritius at 1.5°C for 4 weeks and 20°C for 3 days).

Table 4.2. Decay in litchi fruit during storage at 5, 8, 10, 13 and 20°C.

Storage time (days)	Experiment 1				Experiment 2			Experiment 3		
	5°C	8°C	10°C	13°C	5°C	13°C	20°C	5°C	13°C	20°C
Day 0	1	1	1	1	1	1	1	1	1	1
Day 1	1	1	1	1	1	1	1	1	1	1
Day 3	1	1	1	2	1	2	2	1	2	2
Day 7	1	1	1	2	1	2	2	1	2	2
Day 10	1	1	1	2	1	2	3	1	2	3
Day 13	1	1	2	2	1	3	4	1	3	4

1 = no incidence
2 = one spot to 5% of disease on each fruit surface
3 = 10% on fruit surface
4 = 15% on fruit surface

4.5 Conclusion

The investigation described in this chapter clearly implies that a low storage temperature of 5°C maintained higher sugar, organic acid and total phenolic contents in aril tissue and higher anthocyanin and total phenol concentrations in pericarp tissue in SO_2 fumigated litchi fruit cv. Mauritius during 13 days storage as compared with 8, 10, 13 or 20°C storage. Anthocyanin in fruit stored at 5°C was higher than other treatment resulting in brighter and less brown colour in fruit pericarp. Results confirmed that 5°C was the optimum temperature for storage and distribution of cv. Mauritius litchis and thus acts as the basis for subsequent chapters.

CHAPTER FIVE

Altered physiology and biochemistry of imported litchi fruit held under different vapour pressure deficit

5.1. Abstract

The effects of vapour pressure deficit (VPD) on litchi fruit quality have not yet been fully defined. The aim of this study was to detail the changes in physiology, sugars, organic acids and individual anthocyanin concentrations in imported litchi fruit held at various controlled relative humidity (RH) and VPD levels. SO_2 fumigated (but non acid-treated) litchi imported from Thailand (cv. Kom) and from Israel (cv. Mauritius), were air freighted to the UK and then stored for 9 days at either 5 or 13°C to simulate shelf life conditions. Fruits were stored under a series of controlled RH conditions for the duration of the trial using different concentrations of glycerol in deonised water. Respiration rate and weight loss of both fruit lots were greater in litchi stored at 13°C and VPD of 0.274 kPa. At 5°C and VPD of 0 or 0.042 kPa, sugars and organic acids in aril and pericarp tissue and individual anthocyanins in pericarp were better maintained. This is the first piece of work that has systematically evaluated the effect of a series of VPDs on litchi fruit biochemistry such that implications for designing systems to better maintain visual appearance of imported litchi fruit are discussed.

5.2. Introduction

Although litchi (*Litchi chinensis* Sonn.) fruit has been supplied and consumed world-wide for decades, pericarp browning and dehydration still persist as major postharvest problems. Storage at low temperature can slow down fruit metabolism, and also the rate of growth and spread of pathogens, thus prolonging shelf life. However, there is still a lack of detailed information concerning the effects of other storage parameters on storage disorders in litchi. Vapour pressure deficit (VPD) in the storage environment is an important factor in influencing postharvest life of fresh produce. The VPD is defined as the difference between moisture content in fruit tissue and water vapour in the environment which mainly depends on temperature and relative humidity (RH). However, effects of VPD on the biochemistry of harvested litchi fruit have infrequently been investigated. This might be due partly to the practical difficulties in maintaining defined levels of RH in a storage environment. Air exchange rate, temperature distribution, fresh produce type and packaging material used in the storage room can influence the range of RH encountered (Paull, 1999).

Jiang and Fu (1999) reported the influences of RH on pericarp browning of litchi cv. Huaizhi fruit. They supplied dry (35 %) and wet (>95 %) air streams into storage containers (2.12 m^3) held at 20°C and mixed these air streams to achieve 60, 70, 80 or 90% RH levels. The RH was measured at a position midway between the top of the fruit and the exhaust port by an electrohygrometer sensor calibrated using saturated salt solutions. Litchi stored at 90 % RH showed the lowest water loss, tissue pH level, relative membrane leakage, polyphenol oxidase (PPO) activity and browning scale with higher total anthocyanin content after storage. Relative humidity at 90 % (20°C; humidification method not stated) was also shown to inhibit browning in litchi cv. Hong Huay fruit by limiting water loss, PPO and phenylalanine ammonia lyase (PAL) activities, total anthocyanin and phenol degradation and maintaining higher a* (red colour), followed by the fruit stored at 80 and 70 % RH, respectively (Kaewchana *et al*., 2006).

Although storage at elevated RH has been shown to maintain postharvest quality of litchi, the specific effects of a range of controlled VPD levels on physiological and biochemical changes during storage have not been completely described. Thus, the aim of

this study was to detail the explicit spatial and temporal physiological and biochemical changes in imported litchi fruit as affected by different storage VPDs (temperature and RH), with particular emphasis on sugars and non-volatile organic acids in aril and pericarp tissue, and anthocyanins in pericarp tissue.

5.3. Materials and methods

Sample preparation for Chapter 5 was described in section 3.1.2. The measurement and analysis of weight loss, moisture content, respiration rate, total soluble solids (TSS), pericarp colour, individual sugar, individual organic acid and individual anthocyanin were described in Chapter 3: Methodology.

5.4. Results and discussion

The desired RH conditions of 80, 85, 90, 95 and 100 % RH in containers at 5 or 13°C remained relatively stable during 9 days storage (Figure 5.1). Previous RH studies on litchi have either not stated how RH was controlled (Kaewchana *et al*., 2006) or have used different apparatus capable of defining a series of set levels (Jiang and Fu, 1999). At constant RH, lower temperature resulted in lower VPD level, whilst at the same temperature, higher RH caused lower VPD level (Figure 5.1). Disease was detected on approximately 5 % of surface of fruit stored at 13°C and 100 % RH (VPD = 0.000 kPa) for both cultivars.

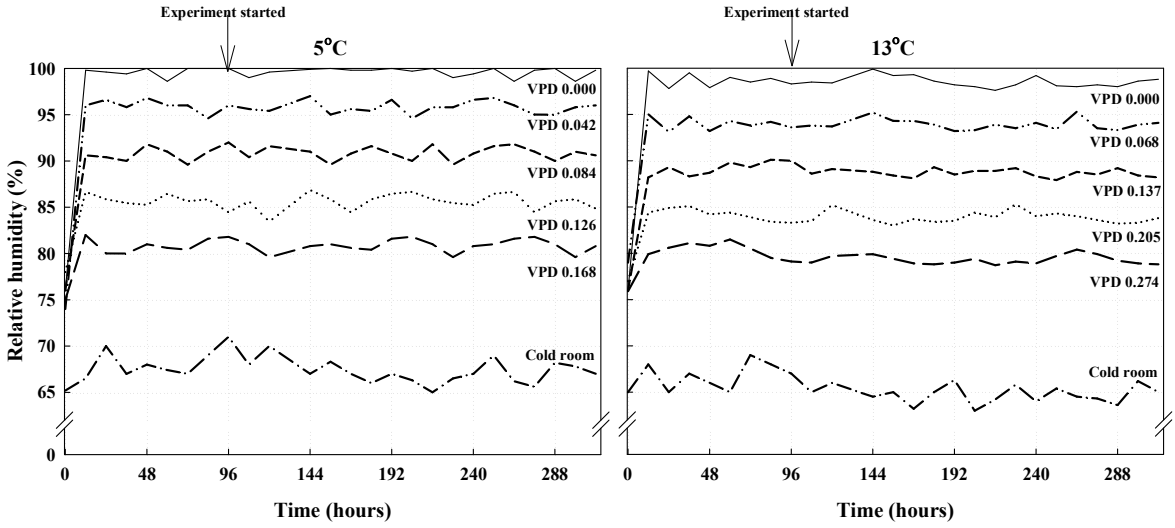


Figure 5.1. Relative humidity levels in the cold room, and inside the storage containers set at 80, 85, 90, 95 and 100 % RH at 5 or 13°C.

5.4.1. Fruit weight loss and pericarp moisture content

Weight loss of fruit from all regimes increased during 9 days storage. Predictably, fruit cvs. Kom and Mauritius stored at 80 % RH and 13°C (VPD of 0.274 kPa) had a significantly higher weight loss than fruit at 85, 90, 95 and 100 % RH, respectively (Figure 5.2). Kaewchana *et al*. (2006) reported that low RH (40-50 %) storage inevitably resulted in greater moisture loss in litchi fruit. In case of litchi fruit, low RH could enhance pericarp micro-cracking (Underhill and Simons, 1993) and damage cellular membranes resulting in an increase in membrane leakage and cellular moisture loss (Huang *et al*., 2005). Temperature significantly affected fruit weight loss, whereby weight loss of fruit stored at 13°C was 1.7-fold (cv. Kom) and 1.9-fold (cv. Mauritius) higher than in the 5°C treatment. Higher temperature causes more free energy of water molecules which increases water movement and potential for exchange to atmosphere around the fruit (Kays and Paull, 2004) resulting in faster evapouration. Higher temperature requires more moisture to saturate air. A greater difference in vapour pressure between the fruit and the storage atmosphere therefore exists and leads to rapid moisture loss from fruit to the environment (Wilss *et al*., 2007). According to the present study, for instance, the air at 13°C and 90 % RH regimes (VPD = 0.137 kPa) would have been drier than the air at 5°C and 90 % RH

(VPD = 0.084 kPa) resulting in faster fruit weight loss. The results indicated that higher moisture loss was induced by lower RH and higher temperature (VPD = 0.274 kPa). There were no significant differences in moisture contents of pericarp between RH treatments in both experiments (Appendix A: Table A83-A86).

5.4.2. Respiration rate

Kom and Mauritius fruit stored at 100 % RH at both 5 and 13°C (VPD = 0.000 kPa) had the lowest respiration rate whilst the highest rates were recorded at 80 % RH (VPD = 0.168 and 0.274 kPa at 5 and 13°C, respectively). Water stress (63 and 70 % RH) was reported to accelerate respiration in litchi fruit cv. Heiye (Peng and Cheng, 2001) and senescence in cv. Huaizhi (Huang *et al*., 2005). However, the impact of a wide range of controlled RH conditions on respiratory activity has not been reported. Unsurprisingly, storage temperature significantly affected the respiration rate, whereby fruit stored at 5°C had a significantly lower respiration rate than fruit held at 13°C (Figure 5.2). A decline in respiration rate was found in fruit from all treatments over storage time. A slight increase in respiration rate for both experiments kept at 13°C was detected after day 6, which could be partially due to pathogenic rot (but less than 5 % of fruit surface) but is more likely to have been influenced by browning (Chen *et al*., 1987; Zhang and Quantick, 2000) since fruit held at 80 % RH and 13°C showed no disease but the most severe browning. The results indicated that higher VPD (low RH and high temperature) encouraged greater fruit desiccation and moisture loss and accelerated respiratory rate during storage time which was in agreement with previous work on litchi cv. Heiye (Peng and Cheng, 2001).

5.4.3. Pericarp colour

Storage temperature, RH, storage duration or their interactions did not affect pericarp colour of litchi cv. Mauritius but did influence cv. Kom. Kom fruit kept at 5°C had significantly higher L* than those at 13°C. The L* and C* of pericarp of cv. Kom fruit stored at 5 or 13°C with 100 % RH (VPD = 0.000 kPa) were significantly higher than with 95, 90, 85 and 80 % RH, respectively. However, fruit kept at 80 or 85 % RH and 5 or 13°C had higher h° (more brown) than those held at 90, 95 and 100 % RH. The results indicated

that pericarp browning was most reduced at 5°C and at 90-100 % RH (VPD = 0.000-0.084 kPa) storage. Values of L*, C* and h° of cv. Kom fruit significantly decreased during 9 days storage (Figure 5.3). The decrease in h° recorded in the present study is in disagreement with previous work by Kaewchana *et al*. (2006) who reported a very slight increase of h° (220 to 225) in stored litchi cv. Hong Huay held at 20°C and 50-90 % RH between days 0 and 9, yet it is unclear whether these fruit were acid dipped.

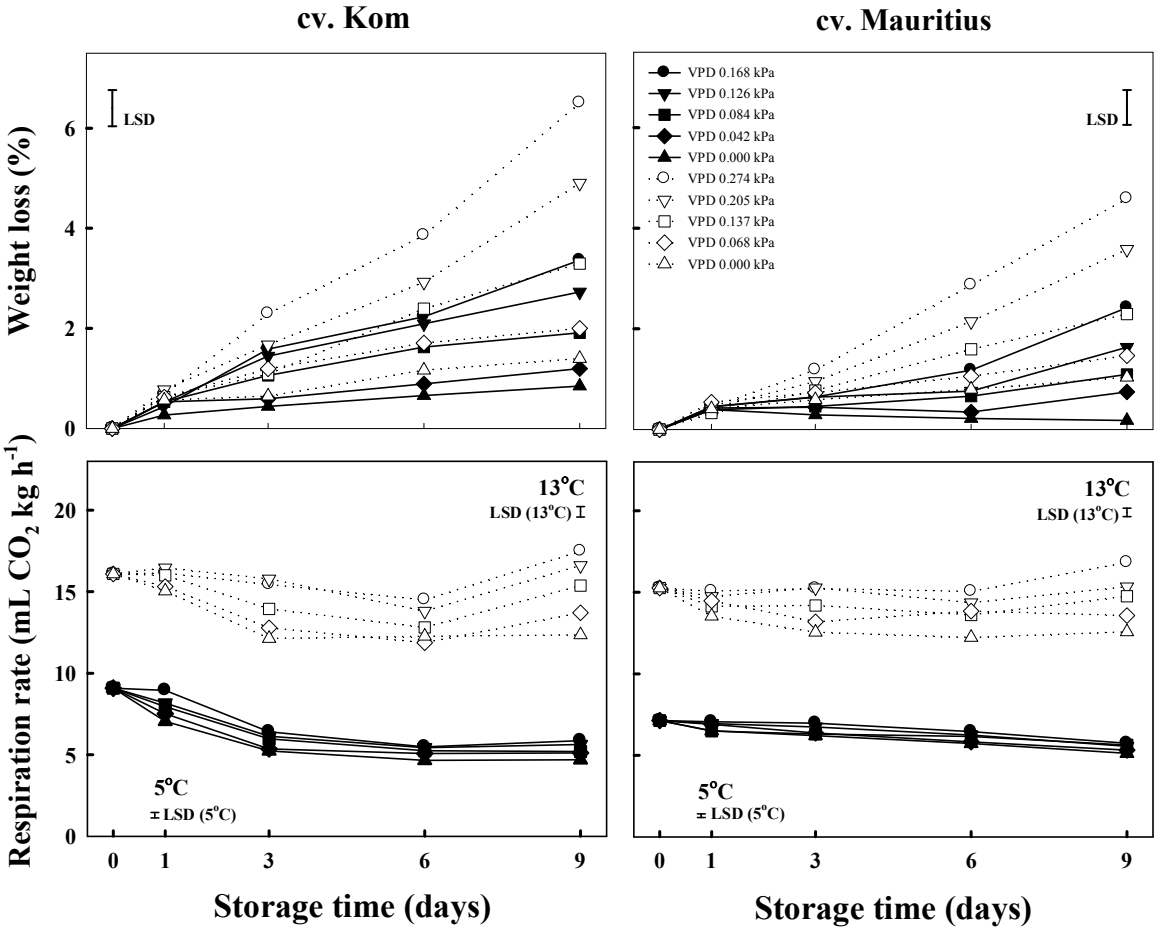


Figure 5.2. Respiration rate (mL CO_2 kg h^{-1}) and weight loss (%) in litchi cvs. Kom and Mauritius stored at 5°C: 80 (●), 85 (▼), 90 (■), 95 (♦) and 100 (▲) % RH and at 13°C: 80 (○), 85 (∆), 90 (□), 95 (◊) and 100 (Δ) % RH during 9 days storage. (LSD; *P<0.05*)

5.4.4. Total soluble solids and sugars concentration

Storage RH, temperature, time or their interactions did not affect TSS in cvs. Kom and Mauritius which may be due to the fact that only slight changes in refractometric values occur in harvested litchi fruit (16.2-19.4 %). Small changes in refractometric values were reported in litchi cvs. Da Zao, Hei Ye, Bengal, Huai Zhi (Batten, 1989) and Mauritius (Chapter 6) during storage. The freeze-dried aril tissue of cvs. Kom and Mauritius mainly contained fructose (368.7 and 252.9 mg g^{-1} DW) followed by glucose (363.6 and 230.3 mg g^{-1} DW) and sucrose (193.8 and 221.5 mg g^{-1} DW). Aril sucrose in both cultivars stored at 5°C and 90 % RH (VPD = 0.084 kPa) was considerably higher than for other RH regimes while the lowest levels were found at 13°C and 80 % RH (VPD = 0.274 kPa). In contrast, all fruit held at 80 % RH, irrespective of temperature, had the highest glucose and fructose levels. Fruit stored at 5°C from both cultivars had significantly higher sucrose and lower glucose and fructose contents than those kept at 13°C (Figure 5.4A and 5.4C). Fructose and glucose concentrations generally increased during 9 days storage time whereas sucrose decreased. These results may be explained by hydrolysis of sucrose to form fructose and glucose together with a probable increase in sugar inverstase enzyme activity during storage (Chan *et al*., 1975). Enhancement of glucose and fructose and deterioration of sucrose during storage time were found in litchi cvs. Hei Ye, Chen Zi (Paull and Chen, 1987) and Rose (Shah and Nath, 2008) and may affect perception of sweetness. However there was no correlation between TSS and aril sugars in either cultivars which is in agreement with Chapter 6.

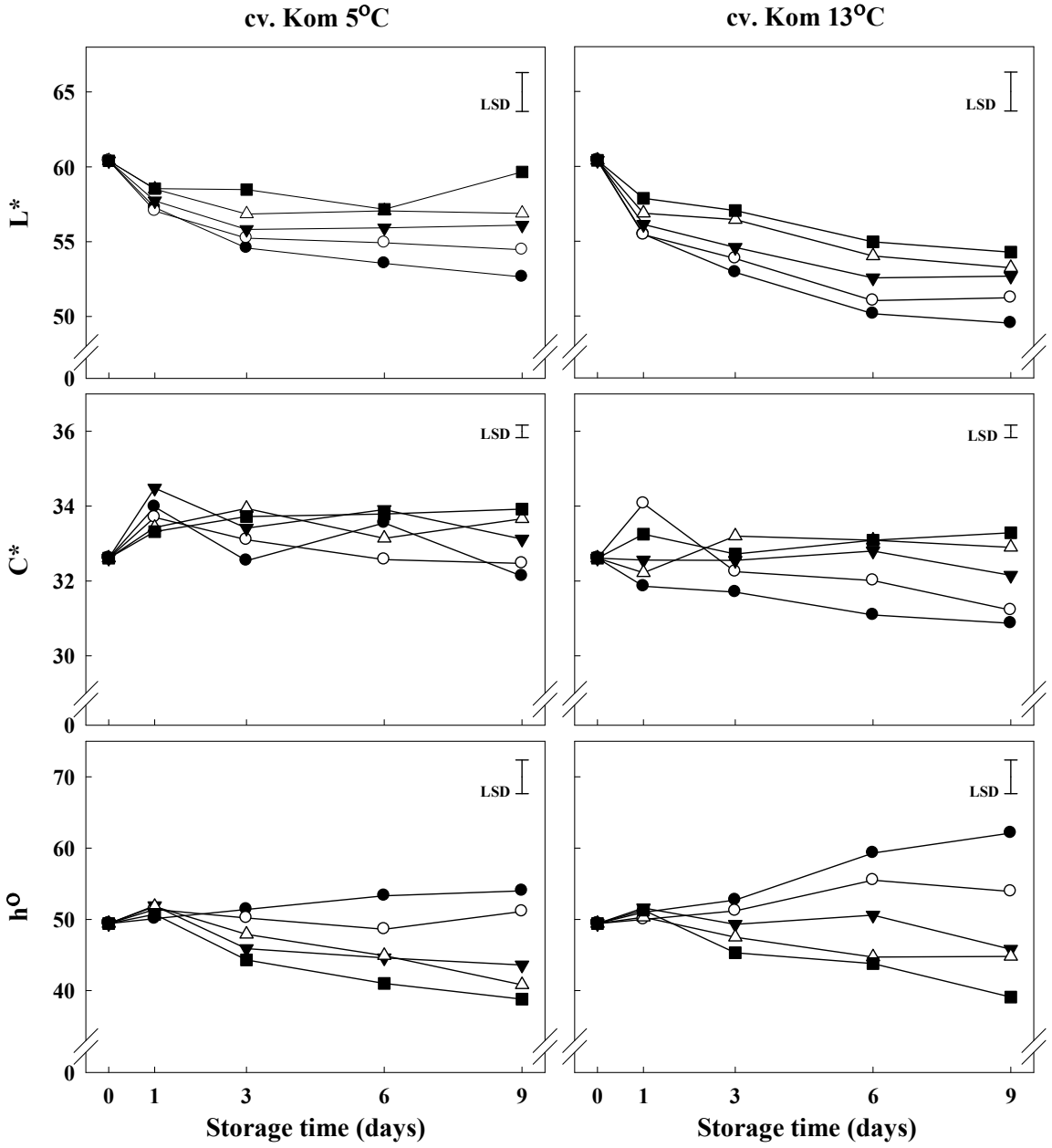


Figure 5.3. Lightness (L*), chroma (C*) and hue angle (h°) of litchi cv. Kom fruit stored at 80 (●), 85 (○), 90 (▼), 95 (Δ) and 100 (■) % RH at 5 or 13°C for 9 days (LSD; *P<0.05*).

Major sugars in pericarp of cvs. Kom and Mauritius were glucose (42.17 and 26.15 mg g^{-1} DW), mannose (39.00 and 28.66 mg g^{-1} DW) and fructose (30.90 and 28.87 mg g^{-1} DW) with trace amounts of sucrose. This is the first study to report the presence and abundance of these non-structural carbohydrates in litchi pericarp. Both cultivars kept at 5°C had higher sugar concentrations than those at 13°C during 9 days (Figure 5B and 5D). Fruit cv. Kom contained 1.56-fold higher glucose and 1.36-fold higher mannose than cv. Mauritius whereas fructose levels did not differ. A high proportion (%) of mannose content in combination with other polysaccharides was reported in litchi pericarp cv. Huaizhi as a strong antioxidant source (Yang *et al*., 2006). Yet, the concentration of mannose in litchi pericarp has not been previously reported. Glucose and fructose concentrations were significantly different according to RH treatments. The RH of 80 and 85 % resulted in lower glucose and fructose concentrations in both cultivars fruit pericarp than 90-100 % RH. Higher moisture content in fruit pericarp at 90-100 % RH could partially accelerate sucrose inversion, by the action of acid (low pH) or invertase enzyme, to form glucose and fructose (Schallenbuger, 1993) resulting in trace levels of sucrose in the present study. Although a decrease in glucose was recorded with increases of mannose and fructose concentrations in non-acid treated and SO_2-free litchi fruit pericarp cv. Mauritius (Chapter 6), the mannose content in the present study remained stable over time (Figure 5.4B and 5.4D). This could be because SO_2 residue in the pericarp was hydrolyzed to sulphurous acid (Bridle and Timberlake, 1997) leading to inappropriate conditions for glucose-mannose-fructose transformation according to Lobry de Bruyn-Alberda van Ekenstein epimerization (Angyal, 1997). Although fruit dehydration did not affect sucrolytic enzyme activities (Lafta and Fugate, 2009) or gluconeogenesis (Costantini *et al*., 2006), increase of fruit weight loss (cv. Mauritius) correlated (albeit weakly) to accumulation of glucose and fructose contents (r = 0.61 and 0.69, respectively).

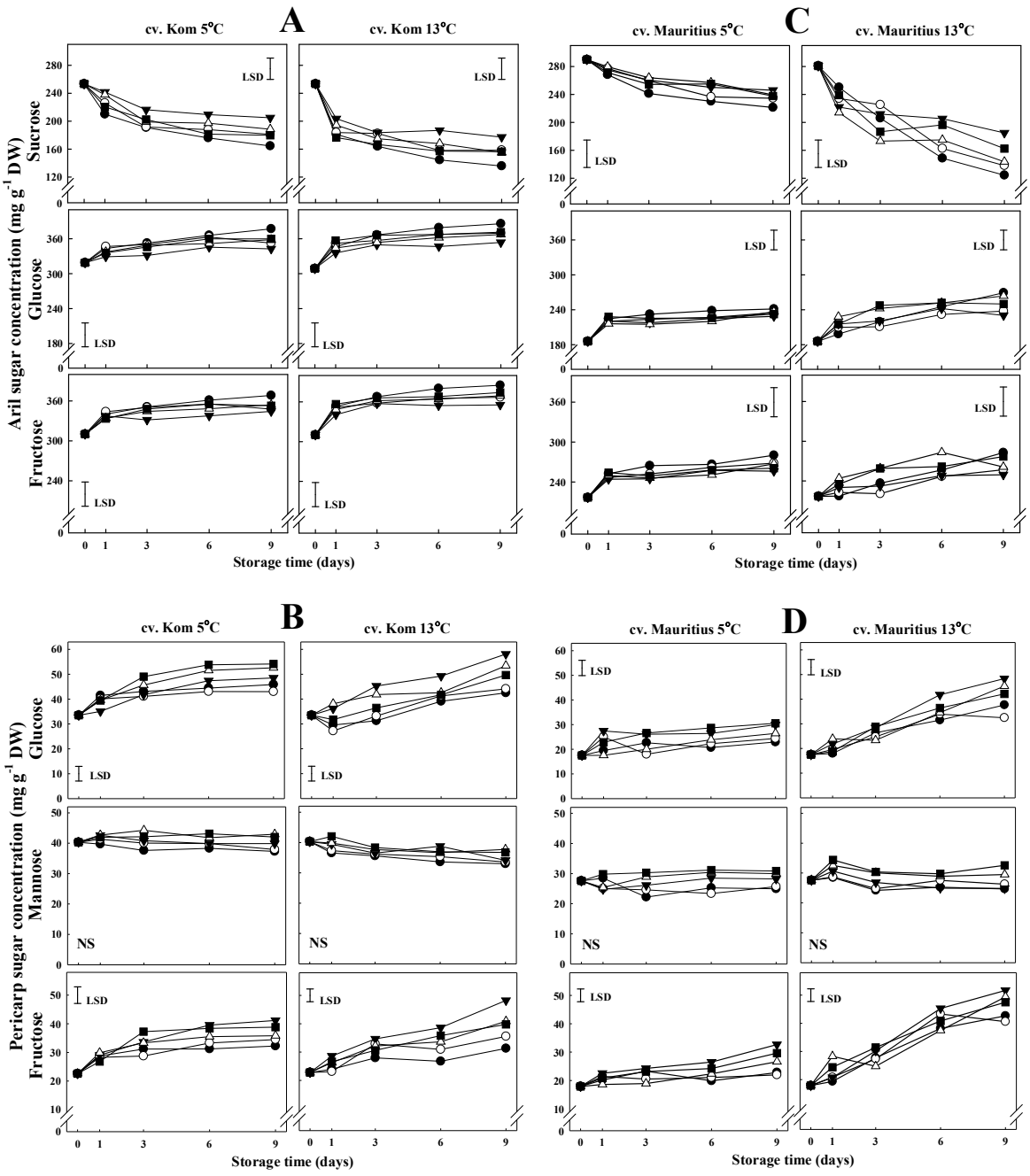


Figure 5.4. Concentrations of sucrose, glucose, mannose and fructose in litchi cvs. Kom aril (A) and pericarp (B), and Mauritius aril (C) and pericarp (D) stored at 80 (●), 85 (○), 90 (▼), 95 (Δ) and 100 (■) % RH at 5 or 13°C during 9 days storage (LSD; *P<0.05*).

5.4.5. Non-volatile organic acids concentration

Malic and tartaric acid (cv. Kom; 22.84 and 16.28 mg g^{-1} DW and cv. Mauritius; 10.27 and 13.37 mg g^{-1} DW, respectively) were the most abundant organic acids found in aril tissue with small amounts of citric, ascorbic and oxalic acids (Figure 5.5). Concentrations of all acids including total acids (malic + tartaric + citric + ascorbic + oxalic acid) in cv. Kom were 1.74-times higher than in cv. Mauritius. Storage RH significantly affected organic acids in aril of both cultivars. Kom fruit at 100 % RH and Mauritius fruit at 95 % RH retained higher malic acid (26.56 and 14.64 mg g^{-1} DW, respectively) than those treated with other RH whereas the highest tartaric acid concentrations were detected at 90 % RH in both cultivars. Fruit stored at 80 % RH contained the lowest concentrations of organic acids in both cultivars. Results indicated that higher RH storage (lower VPD) maintained higher aril organic acids content. Both cultivars fruit stored at 5°C retained higher malic, tartaric, oxalic and total acids than those stored at 13°C. Organic acids are a major source of energy for general metabolism including the respiratory tricarboxylic acid cycle in harvested produce. Lower organic acid contents at higher storage temperature could be due in part to the higher respiratory metabolism. Total organic acid concentrations in both cultivars significantly decreased during 9 days. A similar reduction in acids in aril tissue was also recorded in stored litchi aril cvs. Calcuttia (Nagar, 1994) and Huaizhi (Zhang and Quantick, 2000). Besides, high temperature and low RH storage possibly resulted in an increase in SO_2 movement and absorption in litchi aril (Lemmer and Kruger, 2000) and would lead to off-flavour (not measured) in aril tissue.

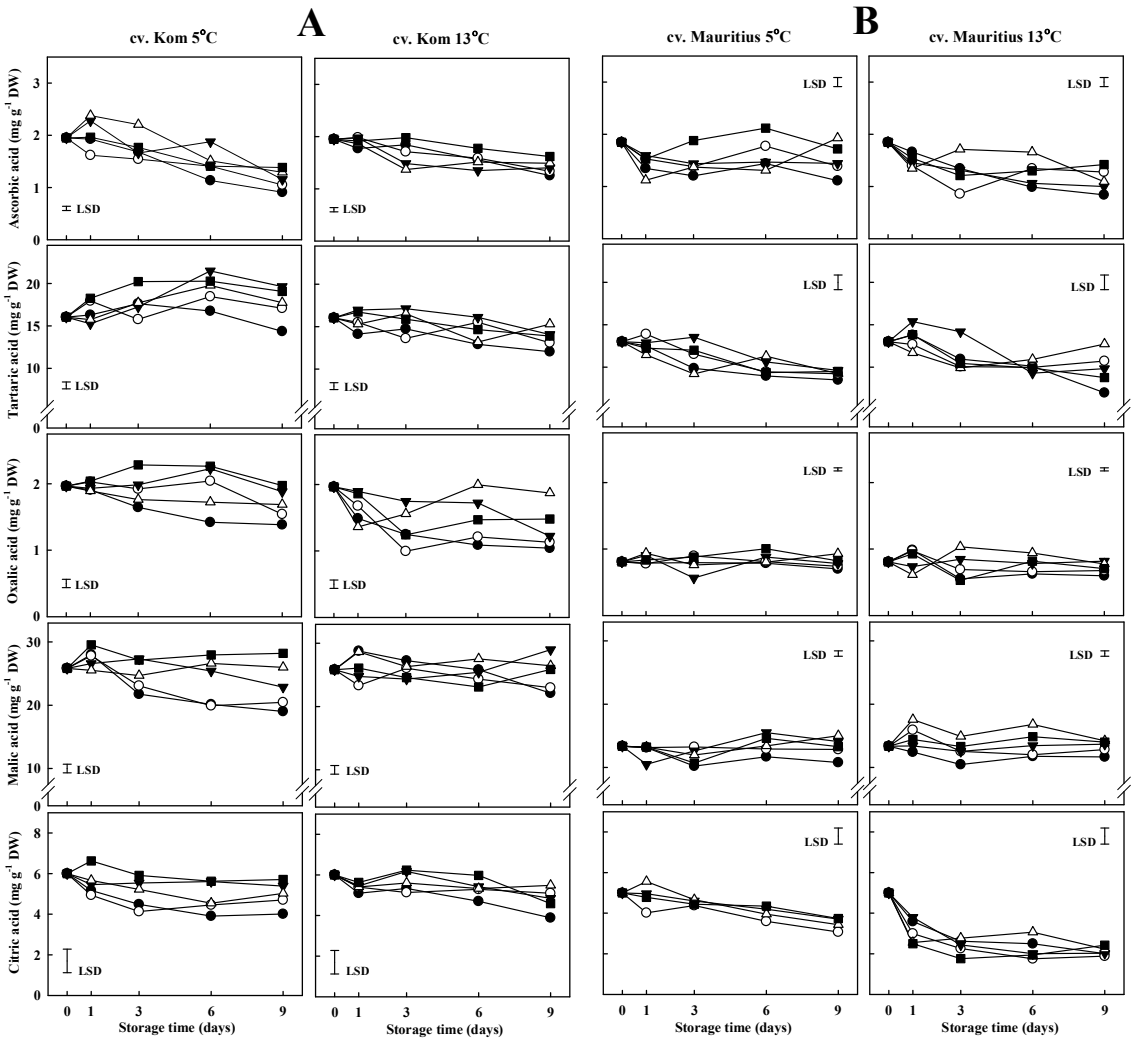


Figure 5.5. Organic acids in aril tissue of litchi cvs. Kom (A) and Mauritius (B) stored at 80 (●), 85 (○), 90 (▼), 95 (∆) and 100 (■) % RH at 5 or 13°C during 9 days storage (LSD; *P<0.05*).

Alteration in organic acid levels is likely to influence pH, and therefore possibly sugar transformation and anthocyanin rutino/glucosides in litchi pericarp. Tartaric, malic and citric acids were the major organic acids found in pericarp of both litchi cultivars. Relatively small amounts of ascorbic and oxalic acids were observed but higher concentrations were generally measured in cv. Mauritius. In general, pericarp acid contents in both cultivars declined over 9 days in agreement with Joubert (1986) and Caro and Joas (2005), but acid levels remained significantly higher in cv. Mauritius at 5°C or 90-100 % RH (VPD = 0.000-0.084 kPa) than other regimes (Figure 5.6). Apart from pericarp endogenous organic acids, SO_2 can also inhibit enzymatic browning by decreasing oxidation and anthocyanin deterioration and increasing membrane integrity (Joas *et al*., 2005; Zheng and Tian, 2006). SO_2 treatment may have slowed down the reduction of pericarp organic acids in the current study which retained higher acid content than in pericarp of non-acid and SO_2-free fruit during storage (Chapter 6). Greater pH changes would have been expected at lower RHs as acid level decreased to a greater extent, especially tartaric and malic acids which were dominant. In addition to the concentration of these two acids being high they have low pK_a (pK_a malic = 3.40 and pK_a tartaric = 2.98) values implying strong acid conditions. Reductions in malic and tartaric acids hence could be possibly linked to an increase in pH in the pericarp of fruit stored at low RH with resultant effects on colour.

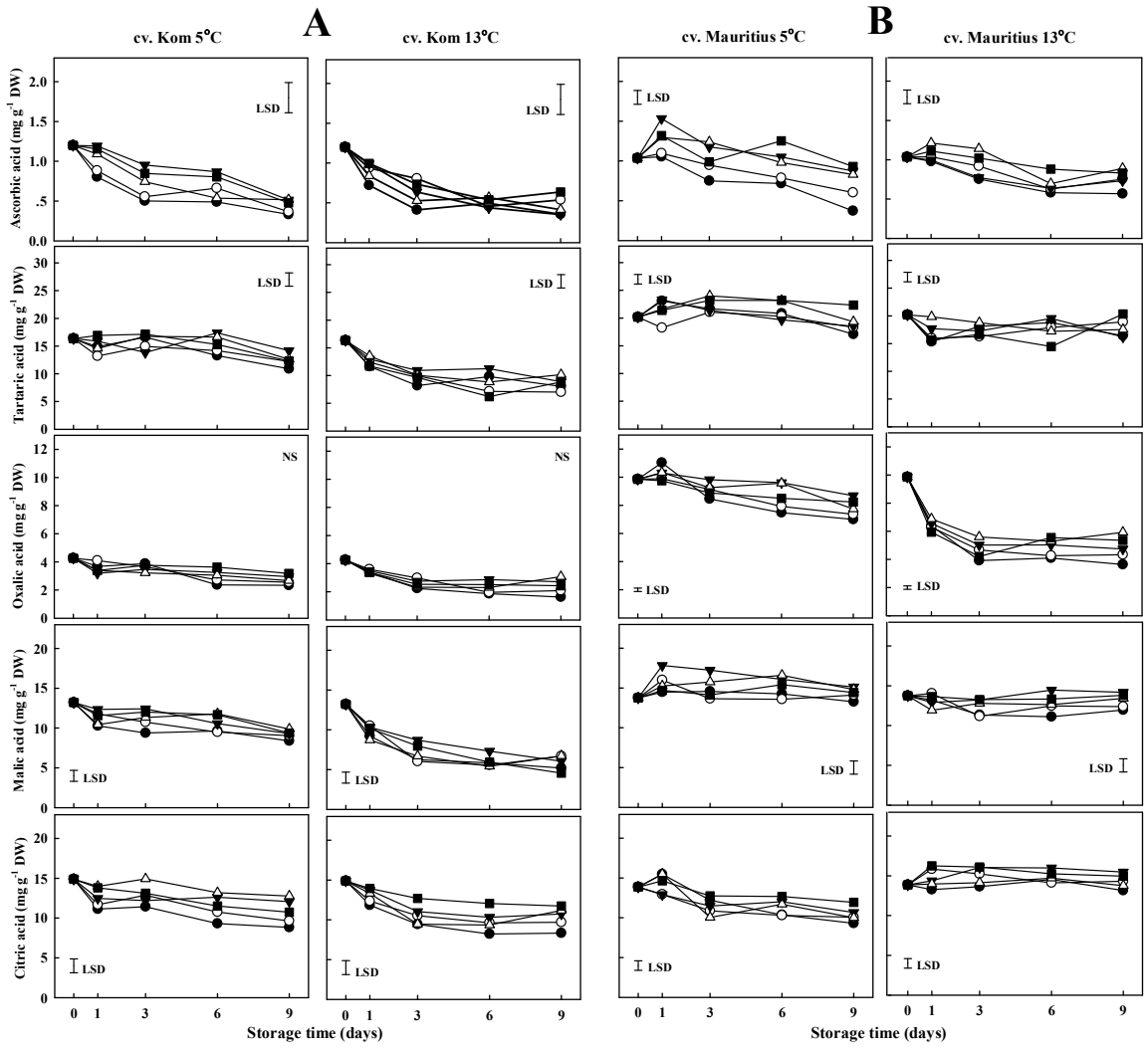


Figure 5.6. Organic acids in pericarp tissue of litchi cvs. Kom (A) and Mauritius (B) stored at 80 (●), 85 (○), 90 (▼), 95 (Δ) and 100 (■) % RH at 5 or 13°C during 9 days storage (LSD; *P<0.05*).

5.4.6. Anthocyanins concentration

The level of individual anthocyanins in litchi cv. Mauritius has been previously detailed (Somboonkaew and Terry, 2010), yet no work to date has described anthocyanins in cv. Kom. Cyanidin 3-rutinoside, cyanidin 3-glucoside and malvidin 3-glucoside were found in the pericarp of both cultivars. The concentrations of cyanidin 3-rutinoside (1678 μg g^{-1} DW) and malvidin 3-glucoside (19.84 μg g^{-1} DW) in cv. Kom were 1.74- and 1.55-times higher than in cv. Mauritius, respectively, whilst cyanidin 3-glucoside in litchi cv. Mauritius was 3.90-fold as compared to cv. Kom fruit (Figure 5.7). Total individual anthocyanin contents of cvs. Kom and Mauritius were lowest under the 80 % RH regime. All anthocyanins in both cultivars generally remained stable or slightly declined over 9 days but were significantly higher in fruit stored at 5°C (Figure 5.7). The decline in anthocyanins may have been accelerated by enzymatic activities of anthocyanin-β-glucosidase (Jiang *et al*., 2006), polyphenol oxidase (PPO) and peroxidase (POD) activity (Huang *et al*., 1990) which can contribute to browning of litchi pericarp. However, SO_2 can prevent these enzymatic browning reactions by being hydrolyzed to colourless chromen-2 (or chromen-4) sulphonic acid (quinine-sulphite complex) which has a similar structure and property to the carbitol form of the anthocyanin (Jurd, 1964; Bridle and Timberlake, 1997). Although anthocyanins in pericarp can be oxidized to anthocyanidin and sugar moieties during storage time, the SO_2 residue interferes with the activities of PPO and anthocyanin inhibiting quinine and melanin formation (Zhang *et al*., 2001) and perhaps pericarp sugar reduction (Figure 5.4B and 5.4D). There are no reports which fully describe the relation between SO_2 concentration in pericarp tissue and browning and/or anthocyanin degradation. The relation between anthocyanin degradation and pericarp discolouration in litchi has been recorded for cvs. Hong Huay (Kaewchana *et al*., 2006) and Huaizhi (Zhang *et al*., 2001), however it is unclear whether these fruit were acid-treated. There was no correlation between pericarp discolouration and anthocyanin deterioration in the present study. The results could be due partly to the belief that visible pericarp discolouration is closely related to senescence–induced anthocyanin transformation rather than its degradation (Underhill *et al*., 1992). Besides the contradiction between fruit colour and colour pigments may be an artifact of the objective measurement system used since it may have been unable to account for the heterogeneity

in colouration of non-acid dipped fruit and the sampling system employed. Redness in stored litchi fruit (25°C, 60 % RH, 48 h) was better correlated with pericarp pH than anthocyanin concentration (Underhill and Crichley, 1994). It is likely that changes in pH will have altered the stability, co-pigmentation and spectra of the anthocyanins found in litchi fruit during storage. As a result and despite anthocyanins being responsible for red pigmentation, the relationship between anthocyanins and litchi pericarp colour is not fully understood.

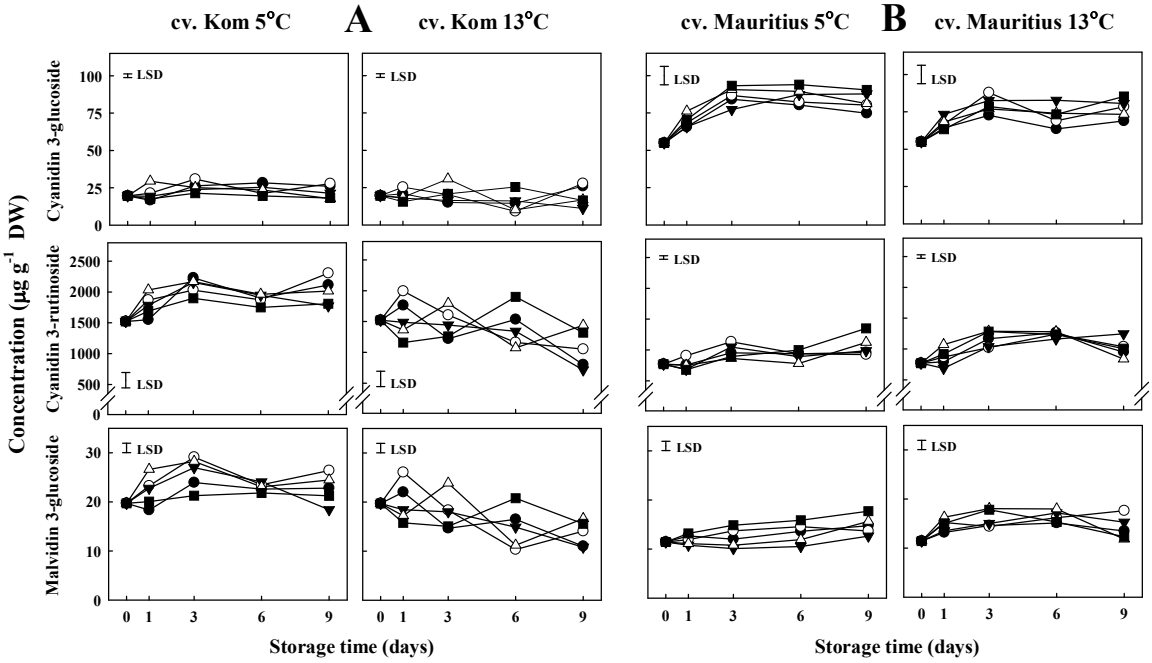


Figure 5.7. Anthocyanins in pericarp tissue of litchi cvs. Kom (A) and Mauritius (B) stored at 80 (●), 85 (○), 90 (▼), 95 (Δ) and 100 (■) % RH at 5 or 13°C during 9 days storage (LSD; *P<0.05*).

5.4.7. Chemometric analysis

Principal component analysis (PCA) of litchi fruit clearly demonstrated the clustering of the samples on PC1 and PC2 (68 and 16 % of the variance, respectively). Cultivar Kom fruit were arranged away from cv. Mauritius fruit along PC1 (Figure 5.8) indicating a different reaction of each cultivar. Fruit cv. Mauritius kept at 5°C and 95-100 % RH (VPD = 0.000-0.084 kPa) were separated from those held at 13°C and 80-90 % RH (VPD = 0.137-0.274 kPa) treatments, respectively along PC1. Although cv. Kom fruit sample could not be differentiated along PC1, the samples were grouped separately into 5°C+80-85 % RH (VPD = 0.126-0.168 kPa), 5°C+90-100 % RH (VPD = 0.000-0.084 kPa), 13°C+80-85 % RH (VPD = 0.205-0.274 kPa) and 13°C+90-100 % RH (VPD = 0.000-0.137 kPa) on PC2. Respiration rate played the most important role in sample separation along PC 1 whilst aril glucose concentration was a key variable for PC2. It is clear, therefore, that RH, temperature and VPD affected not only senescence but also carbohydrate utilization through respiration.

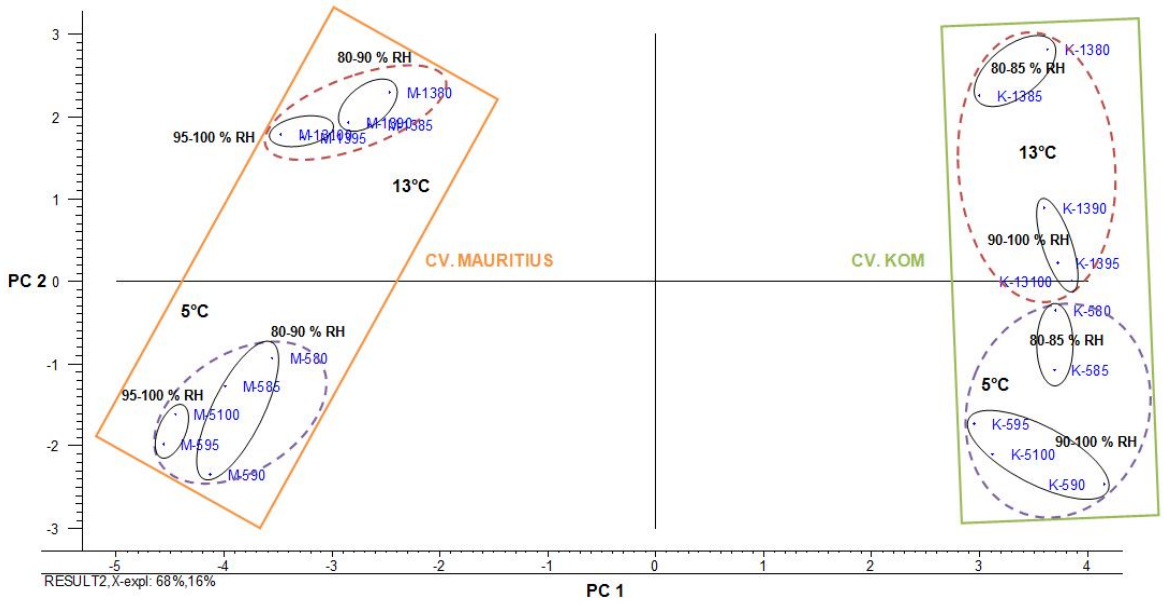


Figure 5.8. PCA bi-plot of litchi fruit cvs. Kom and Mauritius. Clustering of 20 samples from stored fruit at 5 or 13°C and 80, 85, 90, 95 and 100 % RH demonstrated on the loading and score plot of PCA based on the similarities in spatial and temporal variation of weight loss, respiration rate, sugars and acids in aril and pericarp and anthocyanins in pericarp. The outlines of the clusters have been added manually to aid interpretation.

5.5. Conclusion

Results suggested that low VPD (high relative humidity and low temperature) were important to maintain quality of litchi cvs. Kom and Mauritius over 9 days storage. Accompanying reduced weight loss and respiration rate, storage at 95-100 % RH or 5°C (VPD = 0.000-0.084 kPa) significantly controlled the reduction of aril and pericarp sugars and organic acids and retained anthocyanin concentrations. Recommendations are that storage conditions for litchi should not only be centered on maintaining the cool chain but should also consider controlling the VPD at less than 0.068 kPa to attain improved conservation of visual appearance.

CHAPTER SIX

Physiological and biochemical profiles of imported litchi fruit under modified atmosphere packaging

6.1. Abstract

Although sophisticated packaging materials can be used to minimise postharvest changes of litchi fruit, no work has documented the effect of different modified atmosphere packaging on biochemical composition in litchi aril and pericarp tissue. Therefore, the aim of this study was to detail not only the changes in weight and colour, but also individual sugars, organic acids and anthocyanin concentrations using various packaging materials. Non-acid,- and SO_2-free fruit cv. Mauritius, imported from Israel, was packed using four different packaging films *viz*. micro-perforated polypropylene (PP), PropaFresh™ PFAM (PF), NatureFlex™ NVS (NVS), Cellophane™ WS (WS) and unwrapped, and stored at 13°C for 9 days. Concentration of CO_2 and ethylene were greater in WS packs during storage followed by NVS, PF and PP films, respectively. Weight loss of fruit stored in PF film was lower than for other treatments. PF treatment better maintained sugars, organic acids in aril and pericarp tissue and individual anthocyanins in pericarp. These results indicate that PF is the best packaging film to maintain physiological and biochemical properties in litchi fruit.

6.2. Introduction

The time for air freighted litchi to be transported from source to overseas destination can vary between 12 and 15 days. Pericarp browning becomes a major postharvest problem during long-term distribution. Several methods have been used to maintain the quality of fresh litchi fruit. Chemical application, particularly sulfur dioxide (SO_2), has been used to reduce fungal growth, insects, and browning in harvested litchi. However, a surplus of SO_2 can not only induce bleaching of the pericarp and promote off-favour in fruit but also potentially affect consumer health. Residue and application of SO_2 in food have been limited in many regions including Japan, USA and EU. Alternative techniques have, hence, been sought. Packaging technology can maintain postharvest quality of fresh produce when used appropriately. Modified atmosphere packaging (MAP) successfully prolongs shelf life of assorted harvested fruit and vegetables including litchi fruit. The efficacy of MAP is reliant on not only the product but also importantly on the gas permeability and thickness of the polymeric film used since this element plays a vital role in establishing an appropriate atmosphere within the package. Several types of polymeric film have been recently assessed for their ability to reduce litchi pericarp browning, and extend shelf life of litchi fruit (Table 6.1). Harvested litchi fruit quickly lose their red colour and became brown resulting in an unmarketable appearance. Previous works therefore has mostly been concerned with litchi pericarp browning e.g. degradation of anthocyanins by anthocyanase and role of polyphenol oxidase (PPO) and peroxidase (POD) to control the fruit loss. There is a lack of work reporting other biochemical changes in imported litchi fruit.

Litchi is classified as non-climacteric fruit since no ripening occurs after harvest (Nagar, 1994) and stored litchi fruit do not produce CO_2 and ethylene peaks during respiration (Tongdee *et al*., 1982); however there still remains some confusion about whether CO_2 and ethylene have a role in harvested litchi fruit. Apart from pericarp browning and anthocyanins degradation, there is little work which has reported the influences of gas composition on biochemical changes in litchi fruit. Although MAP has been reported to prolong postharvest quality of litchi fruit, the detailed effects of MAP on physiological and biochemical alterations during storage have not been entirely defined or explained. Hence, the aim of this present study was to not only detail the physiological

changes in litchi fruit as affected by different packaging films but also elucidate the affect on sugars, non-volatile organic acids in aril and pericarp tissue and individual anthocyanins in pericarp.

6.3. Materials and methods

Sample preparation for Chapter 6 was described in section 3.1.3. The measurement and analysis of weight loss, moisture content, respiration rate, TSS, pericarp colour, individual sugar, individual organic acid, individual anthocyanin and total phenolic compound were described in Chapter 3: Methodology.

6.4. Results and discussion

6.4.1. Weight and pericarp colour

Plastic films differentially affected weight loss of litchi during 9 days storage at 13°C. Predictably, the unwrapped fruit had the higher moisture loss (Table 6.2). Increases in weight loss over storage time were also recorded in litchi cvs. Mauritius (Sivakumar and Korsten, 2006b) and Kom (Chapter 5). Fruit weight loss, pericarp moisture content and dry matter in all treatments changed depending on the gas permeability of plastic film (Table 6.3). PF film resulted in lower fruit weight loss and thus maintained higher moisture contents in both aril and pericarp, whilst greater weight loss and lower moisture contents were found in NVS-packaged fruit (Table 6.3).

Table 6.1. Effects of modified atmosphere packaging (MAP) on quality of litchi fruit.

MAP Conditions					Cultivars	Storage time (days)	Effects	References
Films	O_2 (kPa)	CO_2 (kPa)	Temperature (°C)	Other treatments				
BOPP	17-18	4-5	2	1-MCP	Mauritius and McLean's Red	21	- PPO, POD and browning ↓ -Anthocyanin and colour ⟷	De Reuck et al., 2009
PP	-	-	1 and 5.5	Antifungal compounds	Not mentioned	40	-Water loss and diseases ↓ - PPO and colour ⟷	Archibald and Bower, 2008
BOPP	16	6	3	Anti-microbial agents	McLean's Red	18	-Decay, PPO activity and browning ↓ -changed red colour to be yellow-orange (h° ↑)	Sivakumar et al., 2008
Xtend®, BOPP	-	-	2	Hot water dip	McLean's Red	34	-BOPP: weight loss ↓retained colour with excellent eating quality -Xtend® caused anaerobic respiration	Sivakumar and Korsten, 2006a
BOPP	17	6	2 and 14	-	Mauritius	36	- Respiration rate and weight loss ↓ - Colour ⟷ and excellent eating quality	Sivakumar and Korsten, 2006b
PE	15-19	2-4	3	CA	Heiye	42	-MAP: colour, anthocyanin and phenols ↓ -MAP and CA did not affect PPO and POD activities	Tian et al., 2005
PE, PVC	-	-	5	-	Hong Huay	12	-PE: weight loss ↑, maintained brighter colour -PVC: maintained better eating quality and less decay	Chiprasart, 2005
Micro-, macro- PE	15	5	1.5	Hot water brushing	Mauritius	28	-Micro-PE: accumulation of CO_2, acetaldehyde and ethanol ↑, inhibited fungal growth and prolong shelf life better than macro-PE	Pesis et al., 2002
PE	-	-	5	-	Hong Huay, Juckapat and Gimjeng	49	-CO_2 and ethylene ↑ while O_2 ↓ -PE did not affect colour, PPO activity, TSS, pH, TA and overall quality	Rattanapanone and Boonyakiat, 2001
PE	-	-	5	Hydro- and room- cooling	Hong Huay	6	-PE with hydro-cooling: weight loss and pericarp browning ↓	Ketsa and Leelawatana, 1992
PVC	-	-	0, 5, 7, 10, 13 and 20	Anti-fungal compounds	Haak Yip and Bengal	40 (0, 5, 7 and 10°C) 19 (13 and 20°C)	-CO_2 ↓ whilst ethylene ↑ -fungal growth with ethylene increased -chilling injury found in fruit stored at 0 and 5°C	Tongdee et al., 1982

BOPP: Bi-axially oriented polypropylene; PE: Polyethylene; PVC: Polyvinyl chloride; CA: Controlled atmosphere; PP: Polypropylene; 1-MCP: 1-methylcyclopropane;
PPO: Polyphenol oxidase; POD: Peroxidase; MAP: Modified atmosphere packaging
Arrow description: ↑ Increase; ↓ Decrease; ⟷ Stable

Average L*, C* and h° in pericarp at day 0 were 49.81, 34.90 and 63.34, respectively. Decrease of L* (darker) and C* (lower red colour intensity) were detected in fruit from all treatments during 9 days storage. Level of h° in PP, PF and NVS treated fruit decreased (deep red colour) over time whilst increase of h° (brown colour) was recorded in unwrapped and WS treated fruit (Table 6.2). The results suggested that unwrapped and WS-treated fruit became darker and turned to deeper brown colour than other treatments. Pericarp discolouration (L*, C* and h°) found in this study were in agreement with the work of De Reuck *et al*. (2009) on pericarp of litchi cvs. Mauritius and McLean's Red fruit. Decrease of L* value was significantly affected by increased fruit weight loss (r = -0.868) and was probably related to the low relative humidity (Chapter 5) within the packages. There was no disease detected during 9 days storage.

Table 6.2. Weight loss, aril and pericarp dry matter, aril and pericarp moisture content, pericarp colour (L*, C*, h°) and total soluble solids of litchi cv. Mauritius fruit in Unwrapped, Perforated Polypropylene, PropaFresh™ PFAM, NatureFlex™ NVS and Cellophane™ WS after 9 days storage at 13°C.

Packaging Materials	Fruit weight loss (%)	Dry matter (%)		Moisture contents (%)		L*	C*	h°	Total soluble solids (%)
		Aril	Pericarp	Aril	Pericarp				
Unwrapped	5.12	17.98	63.95	82.03	36.05	44.20	31.89	61.60	17.92
Perforated polypropylene	2.11	18.18	45.06	81.82	54.94	44.28	33.25	56.48	17.92
PropaFresh™ PFAM	0.37	17.56	37.78	82.44	62.22	47.70	33.62	57.51	17.62
NatureFlex™ NVS	3.16	18.09	49.77	81.91	50.23	45.73	33.27	55.24	17.57
Cellophane™ WS	1.15	17.34	39.50	82.07	60.50	47.90	34.08	67.38	17.48
LSD ($P < 0.05$)	0.197	0.378	1.632	0.378	1.632	3.100	1.426	3.586	0.168
CV (%)	28.2	7.2	11.8	1.6	10.5	9.8	7.3	11.7	6.4

Table 6.3. Water vapour and oxygen permeability of plastic films.

Packaging films	H_2O		O_2	
	Permeability (mol s^{-1} m m^{-2} Pa^{-1})	Test conditions	Permeability (mol s^{-1} m m^{-2} Pa^{-1})	Test conditions
Unwrapped	-	-	-	-
Perforated polypropylene	-	-	-	-
PropaFresh™ PFAM *	3.173×10^{-17}	38°C 90%RH	7.521×10^{-18}	23°C 0%RH
NatureFlex™ NVS *	2.285×10^{-15}	38°C 90%RH	1.410×10^{-20}	23°C 0-5%RH
Cellophane™ WS *	2.348×10^{-15}	38°C 90%RH	1.410×10^{-20}	23°C 0-5%RH

* Innovia Films, 2009

6.4.2. CO_2 and ethylene concentration

Although litchi has been classified as a non-climacteric fruit, high concentrations of both CO_2 and ethylene were detected in this study. WS packs contained higher CO_2 and ethylene contents than other treatments during 9 days followed by NVS, PF, PP and control (Figure 6.1). CO_2 concentration in WS, NVS and PF punnets increased between days 0 and 9 (Figure 6.1A). Greater concentration of CO_2 in WS and NVS film regimes could be due mainly to their low gaseous transmission properties (Table 6.3). Fruit respiratory processes associated with the gas barrier properties of plastic films effectively deteriorated O_2 content (not measured) and enhanced CO_2 accumulation in WS and NVS after 4 days. Elevated CO_2 level in these two treatments could influence the decrease in ethylene concentration (Burg and Burg, 1967). High CO_2 has been found to be a putative inhibitor of ethylene production by repressing 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis and activities of ACC synthase (ACS) and/or ACC oxidase (ACO) (Kubo *et al*., 1996), whilst moderate CO_2 concentration can enhance the ethylene accumulation (Pretel *et al*., 1999). However, CO_2 content in NVS and WS treatments were too high whilst in the PF was an ideal concentration. Very high CO_2 (15-26 kPa) in MAP of litchi fruit cv. Mauritius contributed to accumulation of acetaldehyde and ethanol contents leading to off-flavours in aril tissue (Pesis *et al*., 2002; Sivakumar and Korsten, 2006b). High CO_2 levels combined with moisture in packages may result in production of carbonic

acid and consequent decrease in pH thereby inhibiting microbial growth and decay in packed fruit (Brandenburg and Zagory, 2009).

Ethylene content in WS and NVS treatments rapidly increased between days 0 and 4 of storage whilst a more moderate accumulation over time was found in PF treatment (Figure 6.1B). Gas composition in WS and NVS (first 4 days) and PF (9 days) punnets probably consisted of relatively high O_2 (not measured) and moderate CO_2 concentrations. These compositions of O_2 and CO_2 in MAP can affect the transformation of ACC to ethylene by stimulating activities of ACS and ACO (or ethylene forming enzyme; EFE) (Pearce et al., 1992; Gorny and Kader, 1996; Pretel *et al*., 1999; Kays and Paull, 2004) in ethylene biosynthesis pathway. Ethylene concentration in the present work increased with elevated CO_2 (r = 0.871). However, decrease of ethylene in WS and NVS after 4 days was possibly caused by high amount of CO_2 in those packages (Kubo *et al*., 1996). Enhancement of ethylene levels in PP and PF regimes could be due partially to fruit senescence during storage time (Chen *et al*., 1986). Evolution of ethylene during storage was also observed in stored litchi fruit cvs. Haak Yip, Bengal (trace level – 0.4 μL kg^{-1} h^{-1} at 20°C) (Tongdee *et al*., 1982) and Huaizhi (57.3 – 203.6 nL kg^{-1} h^{-1} at 5°C) (Jiang and Chen, 1995).

6.4.3. Total soluble solids and sugars concentration

TSS content in fruit treated with PF, NVS and WS were significantly lower than in PP and control treatments after 9 days (Table 6.2). This result may be due to CO_2 at ambient level in unwrapped and PP groups leading to faster senescence than other film regimes. Litchi aril contained mainly sucrose, fructose and glucose (324.9, 194.8 and 210.0 mg g^{-1} dry weight (DW), respectively). Glucose and fructose content of fruit from both treatments increased during 9 days whilst sucrose decreased. Similar changes in sucrose, glucose and fructose concentrations during storage were reported in litchi aril cvs. Kom (Chapter 5) and Rose (Shah and Nath, 2008). Fruit wrapped with PF film had higher sucrose and lower glucose and fructose than other films and control (Figure 6.2). Total sugar (glucose + fructose + sucrose) decreased over 9 days but did not differ according to packaging treatment. Although sucrose content has been correlated with TSS in litchi cvs. Hei Ye and Chen Zi (Paull and Chen, 1987), the TSS content in the present study did not

correlate with the concentrations of sucrose, glucose, fructose or total sugars. Lack of TSS-sugar contents correlation could be explained by the restricted change in refractometric values in harvested litchi fruit (16-18%).

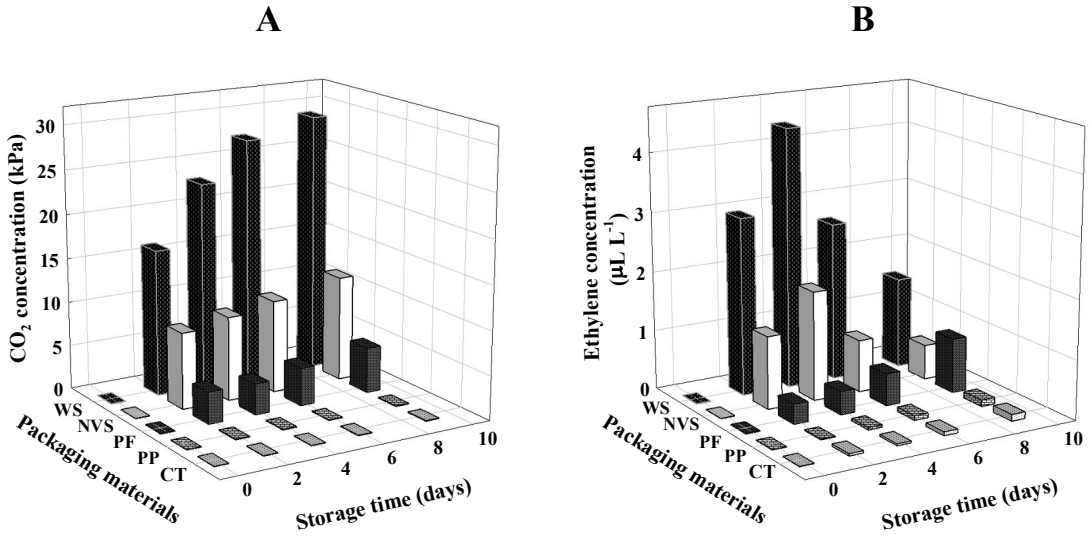


Figure 6.1. Carbon dioxide (A) and ethylene (B) concentrations in unpacked (CT), perforated polypropylene (PP), PropaFresh™ PFAM (PF), NatureFlex™ NVS (NVS) and Cellophane™ WS (WS) films at 13°C during 9 days storage of litchi fruit.

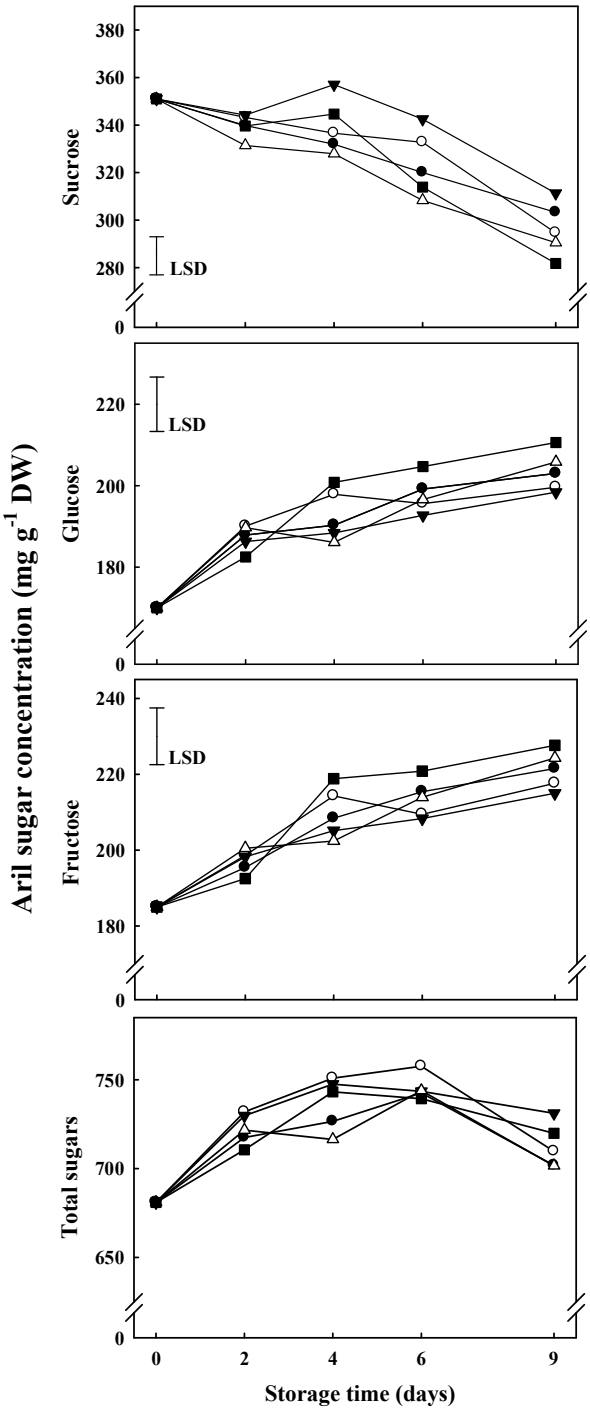


Figure 6.2. Changes of sucrose, glucose fructose and total sugar concentrations in litchi aril cv. Mauritius fruit treated with unwrapped (●), perforated polypropylene (○), PropaFresh™ PFAM (▼), NatureFlex™ NVS (Δ) and Cellophane™ WS (■) films at 13°C during 9 days storage. (P<0.05)

Major sugars found in pericarp of litchi cv. Mauritius were mannose and glucose (40.12 and 35.95 mg g^{-1} DW) with small amounts of fructose (Figure 6.3) and sucrose at trace level. Although high proportion of mannose content in combination with polysaccharide was found in litchi pericarp cv. Huaizhi as a strong antioxidant source (Yang *et al*., 2006), there is no report yet describing the changes in free sugars found in litchi pericarp during storage. In the present study, mannose and fructose in all treatments increased over 9 days whilst glucose decreased. These may be due to glucose being catabolised in glycolysis, anthocyanin glycoside production and/ or epimerised at C-2 position to fructose and mannose structures in the case of Lobry de Bruyn-Alberda van Ekenstein transformation (Angyal, 2001). Glucose can be converted to fructose and/or mannose under alkali conditions (Angyal, 1997), such that higher moisture content and moderate CO_2 level in PF packaged fruit probably led to lower pH (not measured) by increasing carbonic acid. Low pH would not have favoured glucose conversion to mannose and thus maintained higher glucose concentration in pericarp of fruit wrapped in PF film after 9 days. Although CO_2 accumulations in NVS and WS treatments were higher than in PF packages, significantly lower pericarp moisture contents were also observed, and hence this may have been inadequate for accelerating carbonic acid formation.

6.4.4. Non-volatile organic acids concentration

The main organic acid found in litchi aril was malic acid (17.43 mg g^{-1} DW) with low concentrations of oxalic, tartaric, ascorbic and citric acid also recorded (Figure 6.4). Aril total acid (malic + oxalic + tartaric + ascorbic + citric acid) concentration was significantly higher in fruit packed with PF and PP films and generally decreased during 9 days storage. Deterioration of acids content was also observed in stored litchi aril cvs. Calcuttia (Nagar, 1994), Kom (Chapter 5), Huaizhi (Zhang and Quantick, 2000) and Bombay (Mahajan and Goswami, 2004). Organic acids are an important source for general metabolism including the respiratory tricarboxylic acid cycle in harvested fruit (Kays and Paull, 2004).

It is likely that presence and abundance of organic acids in pericarp tissue would also affect pH and thus potentially sugar conversion and anthocyanins. There are no reported studies on the alteration of total organic acid in litchi pericarp during storage. In

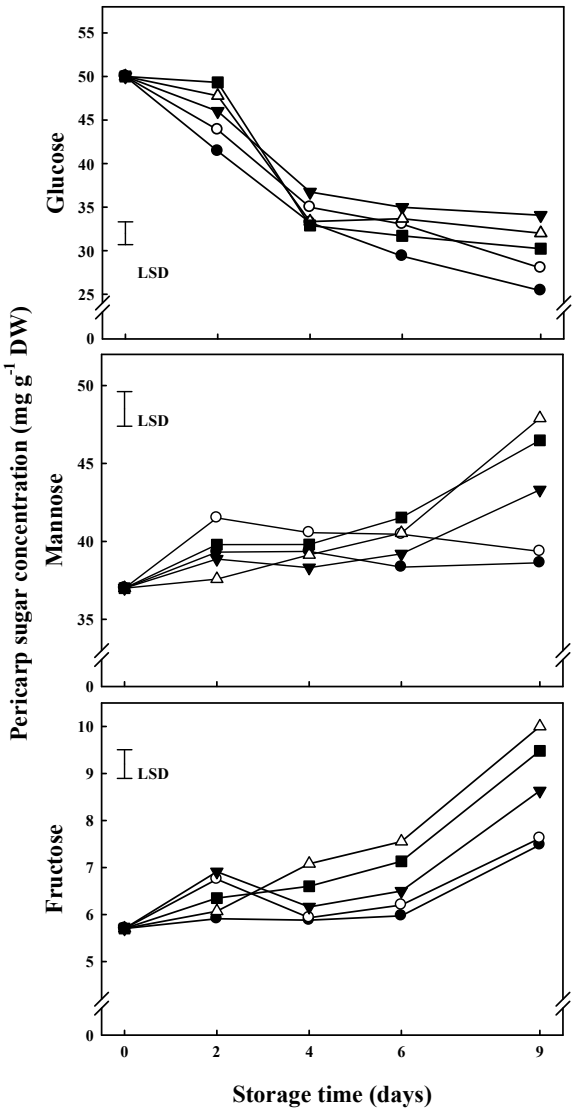


Figure 6.3. Changes of glucose, mannose and fructose concentrations in litchi pericarp cv. Mauritius fruit treated with unwrapped (●), perforated polypropylene (○), PropaFresh™ PFAM (▼), NatureFlex™ NVS (Δ) and Cellophane™ WS (■) films at 13°C during 9 days storage. (P<0.05).

contrast to aril tissue, tartaric acid (20.47 mg g^{-1} DW) was the major organic acid in litchi pericarp followed by citric and malic acid (12.54 and 11.03 mg g^{-1} DW, respectively). Small amounts of oxalic and ascorbic were also detected. In general, total acid contents in litchi cv. Mauritius pericarp decreased over 9 days but were significantly higher in PF treatment (Figure 6.4).

Postharvest exogenous acid and SO_2 treatments account for low pH (below 3) in pericarp and can act as anti-browning treatments in litchi fruit by decreasing oxidation and anthocyanin deterioration, increasing membrane integrity and retaining low POD activity (Joas *et al*., 2005; Zheng and Tian, 2006). Although fruit in the present study were free from acid and SO_2 application, the endogenous acids could work as antioxidant agents against the enzymatic browning reaction resulting in reduction of total amount of organic acid during storage. Decline of pericarp total acid content over time was consistent with works of Joubert (1986) and Caro and Joas (2005). However, there no correlation between pericarp acid content and anthocyanin concentration were recorded in the present work. A minor role of pH level (4.4 - 4.8) on pericarp browning of non-acid treated litchi was also reported in fruit cv. Kwai Mi (Joas *et al*., 2005), despite the organic acids were not measured.

6.4.5. Anthocyanins concentration

Although anthocyanin concentration of stored litchi has been previously reported (Rivera-López, 1999; Zhang *et al*., 2001), this is the first report detailing changes in individual anthocyanins in litchi cv. Mauritius pericarp tissue after storage as affected by different MAP materials. Cyanidin 3-rutinoside (328.00 μg g^{-1} DW) and small amounts of cyanidin 3-glucoside (42.88 μg g^{-1} DW) and malvidin 3-glucoside (5.01 μg g^{-1} DW) (Figure 6.5) were major anthocyanins recorded in litchi pericarp cv. Mauritius. The initial increase in anthocyanins in all packaging treatments was probably due to the transition from ambient air to set equilibrium conditions. The anthocyanins from all fruit decreased during storage except for fruit packed in PF film. Jiang and Fu (1999) documented that pH of litchi pericarp tissue was initially low but increased with pericarp desiccation. Possibly, high moisture content in the PF packaged fruit (free from exogenous acid or SO_2) led to

low cellular pH in pericarp. Highly acidic conditions (below pH 4.5) can stabilise the flavylium salts and anthocyanin structure in

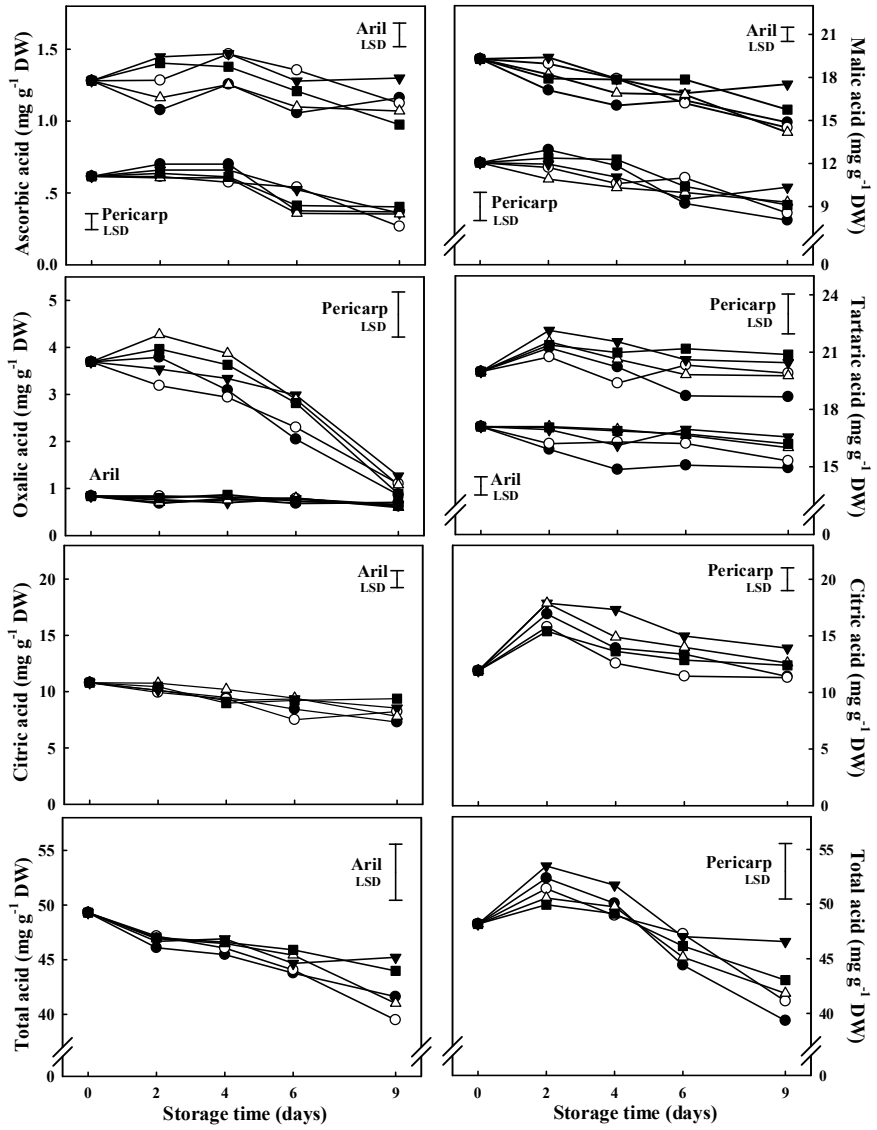


Figure 6.4. Organic acids concentration in litchi aril and pericarp cv. Mauritius fruit treated with Unwrapped (●), Perforated Polypropylene (○), PropaFresh™ PFAM (▼), NatureFlex™ NVS (Δ) and Cellophane™ WS (■) films at 13°C during 9 days storage. (P<0.05)

litchi by decelerating the activity of PPO (Liu *et al*., 2007) and retain anthocyanin concentration during storage.

Fruit from unwrapped treatment (control) showed significantly lower concentrations of all anthocyanins as compared to other packaging materials. The degradation of anthocyanins in unwrapped, PP, WS and NVS treated fruit could be caused by moisture loss disrupting cellular compartmentalisation. This disruption allows PPO located in the vacuole, results in the production of melanin (brown pigments) in litchi pericarp (Underhill and Critchley, 1995) and combined with the coupled oxidation of the red anthocyanins by anthocyanase (anthocyanin-β-glucosidase) (Jiang *et al*., 2004; Jiang *et al*., 2006) to form anthocyanidin and resulting enzymatic browning. However, anthocyanin concentration in the current study was not consistent with L*, C* and h° values.

The role of sugar in litchi pericarp as a strong antioxidant was documented in cv. Huaizhi fruit (Yang *et al*., 2006). Sugar moiety of anthocyanin was found as an inhibitor against the PPO (Jiang, 2000) and POD (Zhang *et al*., 2005) activities in litchi pericarp cv. Huaizhi. The sugar moiety firstly was eliminated from anthocyanin by anthocyanase enzyme producing anthocyanidin and finally PPO and POD led to anthocyanin deterioration.

Additionally, the decrease in anthocyanins between days 2 and 9 in fruit packaging in WS and NVS films may have been affected by elevated CO_2 and ethylene levels. Ethylene has been reported to enhance anthocyanin synthesis by increasing L-phenylalanine ammonia-lyase (PAL) activity, an essential process in the phenylpropanoid pathway in several non-climacteric fruit including strawberry (Jiang and Joyce, 2003), rambutan (Yingsanga *et al*., 2008) and litchi (Zhang and Quantick, 2000). However, high CO_2 level inhibited ethylene production in WS and NVS treatments (Figure. 6.1) which in turn might have reduced PAL activity and eventually anthocyanin concentration (Gil *et al*., 1997; Holcroft and Kader, 1999). There still remains debate over the role of ethylene in so called non-climacteric systems (Terry *et al*., 2007b).

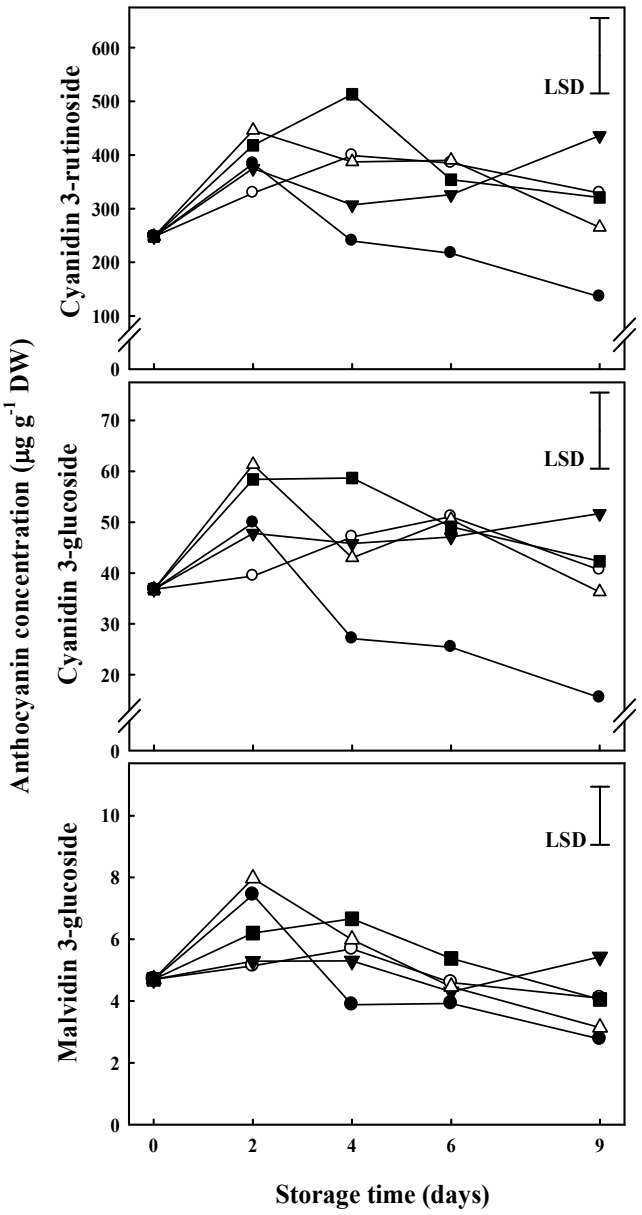


Figure 6.5. Changes of cyanidin 3-rutiniside, cyanidin 3-glucoside and malvidin 3-glucoside concentrations in cv. Mauritius fruit treated with unwrapped (●), perforated polypropylene (○), PropaFresh™ PFAM (▼), NatureFlex™ NVS (Δ) and Cellophane™ WS (■) films at 13°C during 9 days storage. (P<0.05).

6.5. Conclusion

Results indicated that packaging films were suitable to retain better quality of imported litchi cv. Mauritius fruit during 9 days storage as compared to unwrapped fruit. From the different films tested, each plastic film resulted in altered gas composition in the packages and affected postharvest quality. PropaFresh™ PFAM packages significantly reduced sugar transformation and retained anthocyanin content resulting in brighter redness in pericarp as well as limiting fruit weight loss, maintaining anthocyanins, sugar and organic acids in both aril and pericarp.

CHAPTER SEVEN

Influence of temperature and packaging on physiological and biochemical profiles of imported litchi fruit

7.1. Abstract

The aim of this study was to detail the physiological and biochemical changes in non-adulterated and commercially-treated litchi fruit stored in different packaging films under different storage temperatures. Litchi fruit cv. Mauritius treated with either SO_2 and acid (commercially-treated fruit), or free from both SO_2 and acid (non-adulterated fruit), were imported from Israel and packed using two different packaging films *viz*. micro-perforated polypropylene or PropaFresh™ PFAM, or stored unwrapped, at 5 or 13°C for 11 days. Both CO_2 and ethylene concentrations were lower in commercially-treated fruit and at storage of 5°C but higher in PropaFresh™ PFAM films. Weight loss was least in commercially-treated fruit wrapped with PropaFresh™ PFAM at 5°C. Non-adulterated fruit wrapped in PropaFresh™ PFAM had higher individual aril sugars and organic acids whilst commercially-treated fruit retained higher concentrations of anthocyanins. These results indicate that PropaFresh™ PFAM packaging at 5°C could be used to maintain postharvest quality in both commercially-treated and non-adulterated litchi fruit.

7.2. Introduction

Litchi fruit is usually distributed from the growers to the overseas customers in 15 days. Deterioration of the postharvest quality of litchi fruit during distribution could be caused by fruit maturity and senescence (Sharma *et al*., 1986; Huang and Wang, 1990), disease (Huang and Scott, 1985; Sivakumar *et al*., 2008), ethylene exposure and heat and chilling injury (Wong *et al*., 1991; Tongdee *et al*., 1982; Mcquire, 1997). Pericarp discolouration is one of the principal reasons for customer complaints.

The mechanism of litchi pericarp browning has been mainly attributed to polyphenol oxidase (PPO) and high peroxidase (POD) activities (Zhang *et al*., 2005), ascorbic acid oxidation (Jurd, 1972; Jiang, 2000) and degradation of anthocyanins (Huang, Hart *et al*., 1990; Jiang and Fu, 1999). These factors are associated with the aforementioned stresses (e.g. heat injury) and can lead to moisture loss and subsequent disruption of cellular compartmentalisation. Thus, PPO and anthocyanase located in the chloroplasts and other plastids can react with phenolic or anthocyanin substrates located in the vacuole, forming quinine or anthocyanidin which finally results in the production of melanin (brown pigments) in litchi pericarp (Underhill and Critchley, 1995; Jiang *et al*., 2006).

Sulphur dioxide (SO_2) can effectively preclude the incidence of browning in litchi pericarp by transforming to colourless chromen-2 (or chromen-4) sulphonic acid (quinin-sulphite complex) which has a similar structure and property to the carbitol form of anthocyanins (Jurd, 1964; Bridle and Timberlake, 1997). Fumigation with SO_2, therefore, has been widely used to minimise browning in harvested litchi fruit. However, surplus SO_2 can bleach the red colour of fruit pericarp to a pale yellow (Holcroft *et al*., 2005) and cause flavour taint (Rattanachai, 1997).

Bleached pale yellow pericarp can be converted back to a red colour by decreasing the pH of pericarp tissue. Hence, acids have been applied to SO_2 fumigated litchi fruit to ensure the uniformity of pink-red colour in pericarp, despite the fact that it confers an unnatural uniform red colouration. Several studies have reported that acid treatments *viz*. tartaric, ascorbic, citric, phosphoric, oxalic and hydrocholic acids (Jiang *et al*., 2004; Caro and Joas, 2005; Joas *et al*., 2005; Sivakumar and Korsten, 2006; Zheng and Tian, 2006; Ducamp-Collin *et al*., 2008) enhanced red colour in SO_2 treated fruit and minimised litchi

pericarp browning during storage. Although much works have documented the mechanism of pericarp browning in harvested litchi, the mechanism of pericarp discolouration in adulterated fruit (i.e. SO_2 and acid treated) has not yet been widely described. Besides, most studies have emphasised only the physiological or biochemical alterations in litchi pericarp rather than in aril tissue (edible portion). The surplus acid and/or SO_2 might impact consumer health and safety (Tongdee, 1994; Rattanachai, 1997) as an injurious residue and could impact on the biochemical composition in aril tissue. If these are effects are deleterious then they might increase demand for unadulterated fruit in the market.

Modified atmosphere packaging (MAP) is a complementary method for controlling litchi browning and also has been shown to retain postharvest quality of litchi fruit. According to Somboonkaew and Terry (2010), MAP not only resulted in higher anthocyanin content and brighter colour in non-adulterated litchi fruit stored at 13°C but also maintained sugar and organic acid concentrations in both aril and pericarp as well as reducing fruit weight loss during 9 days storage. However, no study has yet fully described the effect of the combination of storage temperature and packaging material on both physiological and biochemical alterations in non-adulterated and commercially-treated fruit. Thus, the aim of this study was to investigate the physiological and biochemical changes in pericarp and aril of commercially-treated and non-adulterated litchi fruit as influenced by different packaging films under two different storage temperatures.

7.3. Materials and methods

Sample preparation for Chapter 7 was described in section 3.1.4. The measurement and analysis of weight loss, moisture content, respiration rate, TSS, pericarp colour, individual sugar, individual organic acid, individual anthocyanin and total phenolic compound were described in Chapter 3: Methodology.

7.4. Results and discussion

7.4.1. Fruit weight loss, pericarp moisture content and colour

Fruit weight loss from all treatments increased during 11 days storage but was 1.21-fold higher in non-adulterated fruit. Lower weight loss was observed in commercially-treated fruit, which was in agreement with results recorded in chitosan-citric or chitosan-tartaric treated fruit cv. Kwai Mi (Joas *et al*., 2005). Unwrapped fruit had a greater fruit weight loss than those wrapped with PP or PropaFresh™ PFAM films (Figure 7.1A). The reduced fruit weight loss in PropaFresh™ PFAM film regimes could be due mainly to the low moisture vapour permeability of this film resulting in slight difference of vapour pressure between fruit and atmosphere in the packages. Similarly, PropaFresh™ PFAM film was reported to minimise weight loss in non-adulterated and SO_2 free litchi fruit cv. Mauritius during 9 days storage (Somboonkaew and Terry, 2010). In the recent study, packaging played a more important role in minimising weight loss of stored litchi fruit than chemical application which was in agreement with Sivakumar and Korsten (2006) who reported that SO_2 or acid treated fruit alone showed greater weight loss than those wrapped with bi-axially oriented polypropylene films. Temperature significantly influenced fruit weight loss, whereby weight loss of fruit stored at 13°C was 1.41-fold and 1.79-fold (for non-adulterated and commercially-treated fruit, respectively) greater than those stored at 5°C. The results indicated that commercially-treated fruit stored at 5°C in PropaFresh™ PFAM film lost less weight during 11 days storage.

Pericarp moisture content in all treatments declined during storage time but was lower in fruit wrapped with PropaFresh™ PFAM at 5°C (Figure 7.1B). Unwrapped fruit at both storage temperatures had significantly lower pericarp moisture content than those wrapped with PP or PropaFresh™ PFAM. Non-adulterated fruit stored unwrapped at 5 or 13°C regimes had lower pericarp moisture content than those commercially-treated fruit. However, wrapping with PropaFresh™ PFAM and PP maintained higher pericarp moisture content in non-adulterated regime than commercially-treated litchi at both storage temperatures (Figure 7.1B). There was a negative correlation between weight loss and pericarp moisture content in all treatments for non-adulterated fruit (r = -0.80) whilst only a weak correlation was recorded for commercially-treated fruit.

The pericarp of non-adulterated fruit in all plastic film treatments had lower L* (darker) and C* (less colour intensity) with higher h° (more brown colour) values than commercially-treated fruit during 11 days storage (Figure 7.2) indicating that the chemical treatment significantly maintained brighter red colour in pericarp of stored litchi fruit,

although this colouration was not natural. Pericarp moisture content was positively but weakly correlated with the discolouration of non-adulterated fruit (r = 0.61) but not with commercially-treated fruit. Pericarp colour of non-adulterated fruit wrapped in PropaFresh™ PFAM film at both storage temperatures maintained a brighter and more red pericarp colour than those fruit wrapped with PP and unwrapped regimes, respectively. Discolouration of fruit pericarp was significantly delayed by 5°C storage rather than 13°C which was consistent with studies on cvs. Kom and Mauritius (Chapter 5 and 6, respectively).

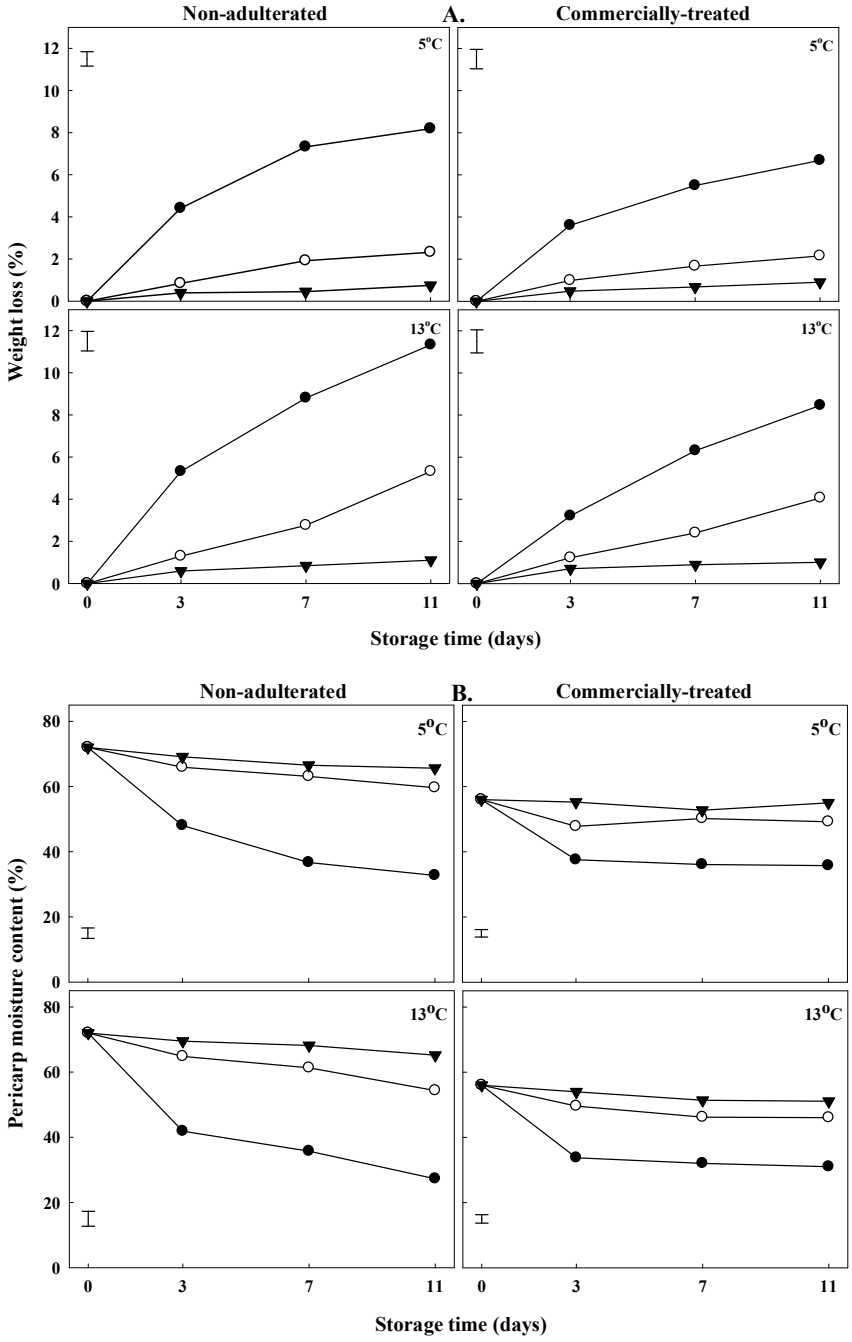


Figure 7.1. Weight loss (A) and pericarp moisture content (B) in litchi fruit stored in perforated polypropylene (○), PropaFresh™ PFAM (▼) and unwrapped (●) at 5 or 13°C during 11 days storage ($P < 0.05$).

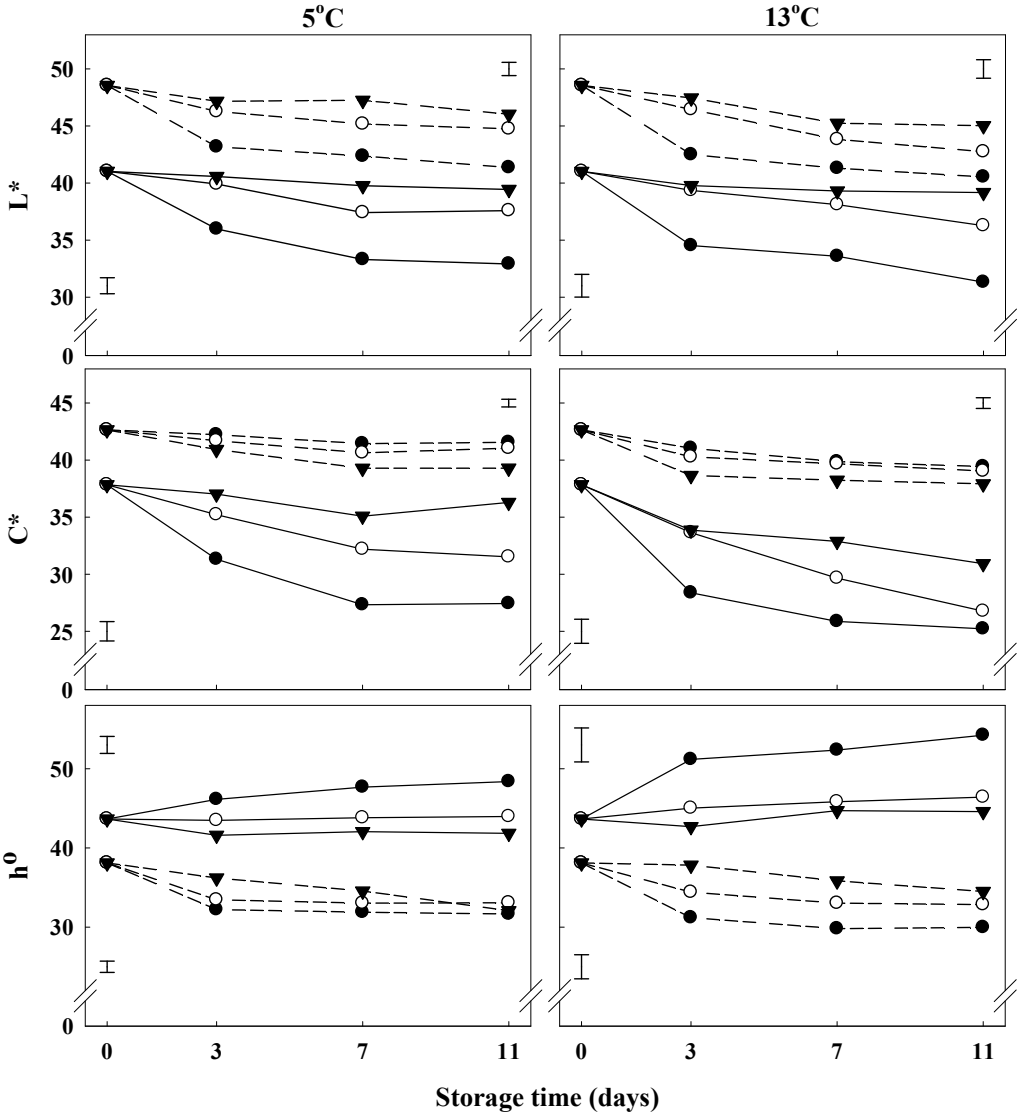


Figure 7.2. Pericarp colour (L*, C*, h°) of non-adulterated (—) and commercially-treated (---) treated litchi fruit stored in perforated polypropylene (○), PropaFresh™ PFAM (▼) and unwrapped (●) at 5 or 13°C during 11 days. ($P < 0.05$).

7.4.2. CO_2 and ethylene concentration

Although litchi has been classified as a non-climacteric fruit, elevated concentrations of both CO_2 and ethylene were detected in this study. The PropaFresh™ PFAM packs contained the highest CO_2 and ethylene levels during 11 days storage, followed by PP packs and unwrapped (Figure 7.3). The CO_2 concentration in PropaFresh™ PFAM wrapped punnets increased between days 0 and 7 and then declined until the end of storage whilst ethylene increased progressively over the time. The concentrations of CO_2 and ethylene recorded in PropaFresh™ PFAM film regimes could be due mainly to their lower gaseous transmission properties in PropaFresh™ PFAM than PP film. Enhancement of CO_2 and ethylene levels in PropaFresh™ PFAM regimes also could be due partially to increase of fruit senescence during storage time. Increases in ethylene concentrations in PropaFresh™ PFAM wrapped litchi punnets over time has previously been reported by Somboonkaew and Terry (2010). Non-adulterated fruit wrapped with PropaFresh™ PFAM film resulted in higher CO_2 and ethylene accumulations than commercially-treated fruit. This could be due to acid impregnation after acid application providing a protective layer on the fruit surface against atmospheric oxygen which may decrease respiration rate and lead to low CO_2 and ethylene production and accumulation in those packages of commercially-treated fruit. The results implied that CO_2 and ethylene affected the deterioration in stored litchi fruit. According to Somboonkaew and Terry (data not published; Appendix B), the ethylene blockers (1-methylcyclopropane; 1-MCP) and ethylene scrubber (palladium promoted powdered materials; Pd) could control pericarp discolouration in non-adulterated litchi fruit during storage at 13°C for 11 days as compared against untreated fruit. Peng and Cheng (2001) reported that hydrochloric acid treated litchi fruit stored at 4°C had a lower respiration rate (mg CO_2 kg^{-1} h^{-1}) than those untreated fruit kept under the storage conditions.

There was no disease detected in any treatments during 11 days storage in the current study. This could be because of low pH in commercially-treated fruit pericarp (not measured) which can suppress microbial growth and decay (Brandenburg and Zagory, 2009). For non-adulterated regimes, moderate CO_2 levels combined with moisture in fruit with PropaFresh™ PFAM packages resulting in production of carbonic acid and consequent decrease in pericarp pH and thus inhibited disease. The low moisture

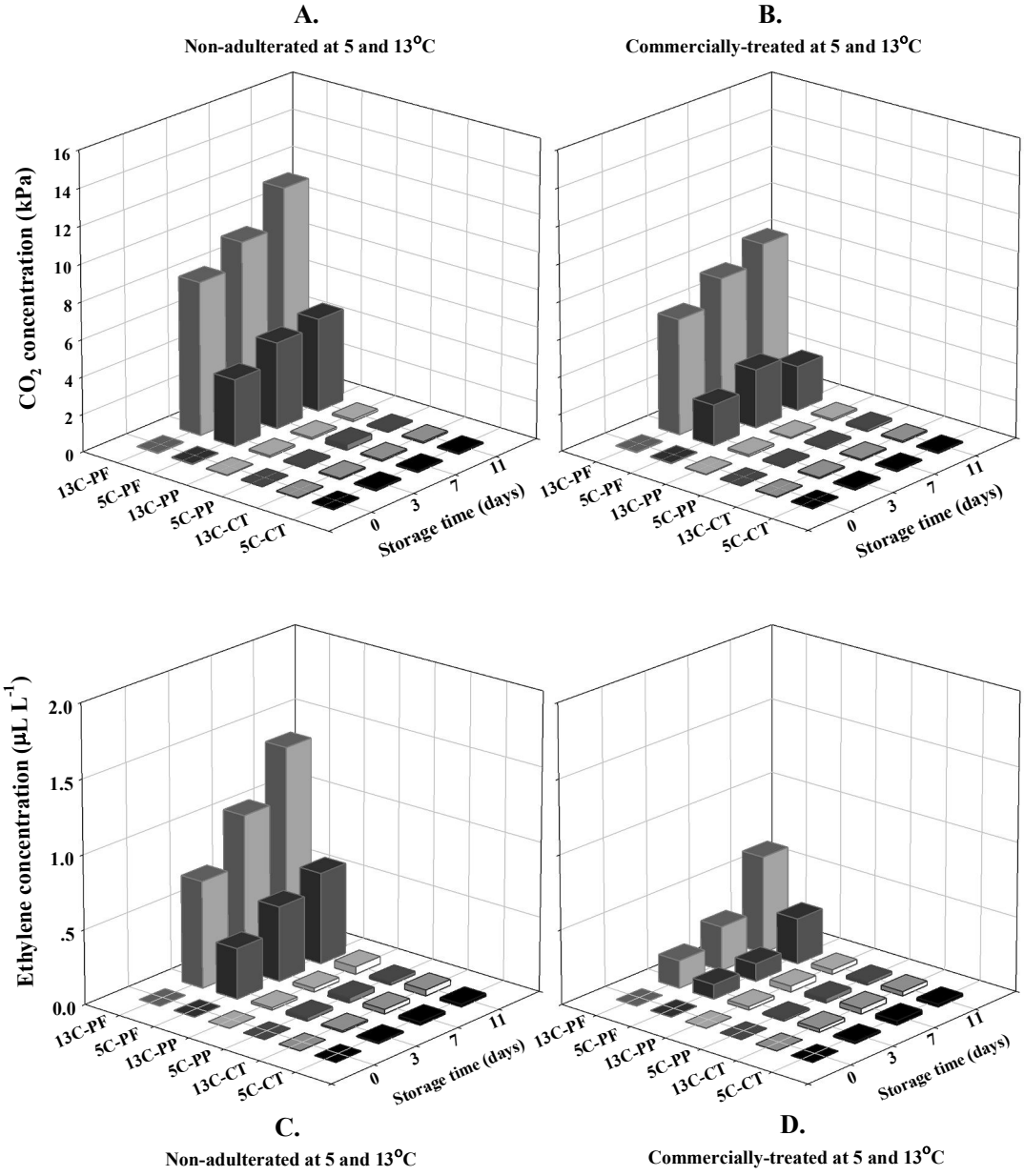


Figure 7.3. Carbon dioxide and ethylene concentrations in micro perforated polypropylene (PP), PropaFresh™ PFAM (PF) and unwrapped (CT) during 11 days storage of non-adulterated (A and C) and commercially-treated (B and D) litchi fruit. Each value is the mean of 3 packs.

content in the pericarp of non-adulterated fruit in PP and unwrapped regimes probably resulted in less suitable conditions for pathogen growth. The current results were in agreement with results in Chapter 6 which reported no disease in non-adulterated fruit stored either in PP or unwrapped at 13°C for 9 days.

7.4.3. Total soluble solids and sugars concentration

Total soluble solids (TSS) were not significantly affected by storage temperature or time. The TSS content in commercially-treated fruit was higher than in unadulterated fruit which was also observed in litchi fruit treated with phosphoric acid (Jiang *et al*., 2004) and citric and ascorbic acid (Na Phan, 2007). Unwrapped fruit maintained higher TSS than those wrapped in PP or PropaFresh™ PFAM films, respectively (Table 7.1). Increases in TSS in stored litchi fruit cv. Mauritius were reported to be minimised by modified atmosphere packaging (Sivakumar and Korsten, 2006b). This could be due partly to the CO_2 concentration being at ambient levels in unwrapped and PP treatments, which accelerated fruit senescence compared with the PropaFresh™ PFAM packaging. Increase of TSS was also possibly caused by fruit water loss which could contribute to higher concentration of soluble solids in stored litchi fruit. The freeze-dried aril tissue of non-adulterated fruit contained mainly sucrose, fructose and glucose (277.9, 237.5 and 212.5 mg g^{-1} dry weight (DW), respectively), whilst fructose and glucose (361.4 and 336.0 mg g^{-1} DW, respectively) were dominant in the aril of commercially-treated fruit with small amounts of sucrose (62.1 mg g^{-1} DW) (Table 7.1). Unadulteration and storage at 5°C resulted in a higher concentration of sucrose with lower glucose and fructose concentrations over storage time. Application of acid and SO_2 to fruit coupled with storage at 13°C may possibly accelerate fruit metabolism including sugar inverstase enzyme activity which epimerises sucrose to glucose and fructose (Chan *et al*., 1975). Transformation of these sugars apparently resulted in higher calculated sweetness (1.0 × sucrose + 0.6 × glucose + 1.8 × fructose; Keutgen & Pawelzik, 2008) in commercially-treated fruit. Higher TSS was recorded in commercially-treated litchi in the current study; however, there was no significant difference in total sugar contents according to plastic film treatments, storage temperature and time. In the present study, there was no

correlation between TSS and sucrose, glucose, fructose or total sugars concentration. This could be due partly to the small range of changes in refractometric level (15.5-18.3%) in stored litchi fruit.

Table 7.1 Total soluble solids (TSS), sugars and organic acids in aril tissue of non-adulterated (N-A) and commercially-treated (C-T) litchi fruit stored in perforated polypropylene, PropaFresh™ PFAM and unwrapped at 5 or 13°C after 0 and 11 days storage.

Packaging	Fruit type	Temperature (°C)	TSS (% Brix)	Sugars (mg g^{-1} DW)			Organic acids (mg g^{-1} DW)		
				Sucrose	Glucose	Fructose	Ascorbic	Malic	Tartaric
All packaging at day 0	N-A	20	17.23	314.3	228.0	220.0	1.30	23.17	11.00
	C-T	20	17.50	115.0	319.0	323.0	0.95	19.25	9.00
Unwrapped	N-A	5	17.00	272.8	207.3	234.5	1.08	13.19	6.23
	N-A	13	16.81	218.3	232.5	276.3	1.05	11.54	6.87
	C-T	5	18.46	41.1	340.7	367.2	0.61	10.54	3.93
	C-T	13	18.83	27.1	347.2	385.6	0.52	9.06	3.76
Micro perforated polypropylene (PP)	N-A	5	16.34	288.3	204.5	226.8	0.86	15.82	6.64
	N-A	13	16.30	250.2	220.3	255.8	0.72	14.46	6.15
	C-T	5	18.32	46.1	347.5	372.5	0.50	11.86	4.14
	C-T	13	17.84	25.7	345.1	379.5	0.46	10.89	3.73
PropaFresh™ PFAM	N-A	5	16.37	296.1	215.9	244.5	0.86	16.10	7.00
	N-A	13	15.53	268.1	215.1	261.5	0.80	15.53	6.25
	C-T	5	17.76	59.2	339.2	364.4	0.50	12.12	4.52
	C-T	13	18.04	31.7	348.7	375.1	0.29	9.77	3.74
LSD ($P < 0.05$)			0.645	12.21	10.01	8.91	0.656	2.148	1.765
CV (%)			8.1	10.8	6.8	5.5	24.5	16.4	18.6

7.4.4. Non-volatile organic acids concentration

Malic acid was the major organic acid measured in freeze-dried aril of both commercially-treated and non-adulterated fruit (15.15 and 12.68 mg g^{-1} DW, respectively; Table 1) with moderate levels of tartaric, citric, oxalic and ascorbic acids. Total acid (malic + tartaric + citric + oxalic + ascorbic acid) content in non-adulterated fruit was significantly lower than in commercially-treated fruit. Higher titratable acidity (TA) was also found in the aril of litchi fruit treated with hydrochrolic (Jiang *et al.*, 2004) or phosphoric acid (Sivakumar and Korsten, 2006b), whilst citric (Na Phan, 2007) and oxalic

acid (Zheng and Tian, 2006) dips did not influence TA in litchi aril. The increase in total acid content or TA in treated fruit could be due to transfer of exogenous acid through pericarp to aril tissue which leads to off-taste and flavour in the aril (Ducamp-Collin *et al*., 2008). Storage temperature significantly affected organic acids in aril of both commercially-treated and non-adulterated fruit, where fruit stored at 5°C had higher levels of malic, tartaric, citric and total acids (14.62, 6.18, 5.30 and 29.32 mg g^{-1} DW, respectively) than those stored at 13°C. There was no difference in organic acids concentration according to the plastic film treatments.

7.4.5. Anthocyanins concentration

Cyanidin 3-rutinoside, cyanidin 3-glucoside and malvidin 3-glucoside were found in the pericarp of both chemically-treated and non-adulterated fruit. Although higher concentrations of cyanidin 3-glucoside (100.2 µg g^{-1} DW) and malvidin 3-glucoside (12.54 µg g^{-1} DW) were detected in non-adulterated fruit, commercially-treated fruit in the current study contained higher cyanidin 3-rutinoside concentrations (1037 µg g^{-1} DW) which resulted in higher total anthocyanins in commercially-treated fruit. Cyanidin 3-rutinoside was more stable under chemical application and low storage temperatures (Rubinskiene *et al*., 2005) than the other two anthocyanins analysed. In unwrapped fruit, cyanidin 3-glucoside, cyanidin 3-rutinoside and malvidin 3-glucoside of non-adulterated fruit decreased by 55.7, 40.8 and 49.3 %, respectively between days 0 and 11, whilst reductions of only 38.6, 26.2 and 31.3 % were found in commercially-treated pericarp. This observation suggests that chemical treatment inhibited the reduction of anthocyanin in litchi pericarp tissue. Jiang *et al*. (2004) and Zheng and Tain (2006) found that less browning in stored litchi fruit was closely related to inhibition of PPO and POD activities by exogenous acidity in pericarp tissues rather than degradation of anthocyanins. It is likely that the alteration in pH changed the stability, co-pigmentation and spectra of the anthocyanins found in stored litchi fruit (Joas *et al*., 2005). However, Ducamp-Collin *et al*. (2008) reported that all individual anthocyanin concentrations in chitosan-citric treated litchi fruit cvs. Kwai May and Wai Chee were lower than those in non-adulterated fruit due to an increase in PPO, POD and anthocyanase activities over storage time. This inconsistency may be because of the use of different fruit cultivars, acid types, postharvest

treatments and storage conditions. In the recent study, fruit from all treatments stored at 5°C contained higher concentrations of all anthocyanins than those held at 13°C. However, storage temperature in the current work apparently was not a dominant factor influencing the anthocyanin alteration as compared to the chemical (SO_2 and acid) treatments (Figure 7.4). Wrapping fruit with PropaFresh™ PFAM film resulted in a higher concentration of all anthocyanins than wrapping with PP or no wrapping during 11 days storage. The lower content of all anthocyanins in unwrapped fruit could be due mainly to moisture loss causing disruption of cellular compartmentalisation, which accelerates enzymatic activities to form a brown pigment in pericarp tissue (Jiang *et al*., 2006). However, anthocyanin levels in the present study did not correlate with litchi pericarp colour (L*, C* and h°) of chemically-treated and non-adulterated fruit.

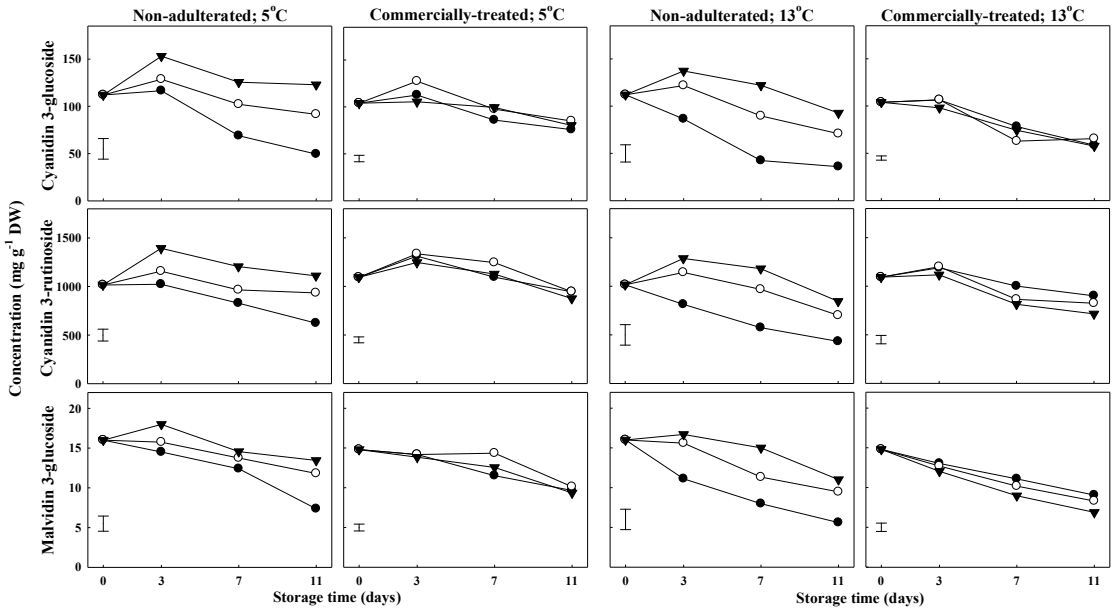


Figure 7.4. Anthocyanin concentrations in pericarp of litchi fruit stored in perforated polypropylene (○), PropaFresh™ PFAM (▼) and unwrapped (●) at 5 or 13°C during 11 days storage. ($P < 0.05$).

7.5. Conclusions

The investigation clearly implies that PropaFresh™ PFAM film and a low storage temperature of 5°C maintained higher sugar and organic acid contents in aril tissue and higher anthocyanin concentrations in pericarp tissue in both commercially-treated and non-adulterated litchi fruit cv. Mauritius during 11 days storage as compared with unwrapped or PP packaged fruit or 13°C storage. Treatment with SO_2 and citric acid decelerated reduction of aril organic acids and anthocyanins whilst promoting aril sugar transformation. Results also suggest that PropaFresh™ PFAM could replace PP packaging as a new active packaging film for litchi industry and be a substitute for chemical treatment to maintain quality of litchi fruit.

CHAPTER EIGHT

General discussion and future recommendations

8.1. Discussion

At least half a million tonnes of fresh litchi are distributed world-wide annually, but litchi producers, importers and exporters are faced with the postharvest problems of decay and pericarp browning which decrease market value. Lack of appropriate postharvest handling is the principal factor leading to fruit of unmarketable quality. For instance, unrefrigerated air storage of fresh litchi after harvest causes drying and browning of the fruit pericarp. Several methods (e.g. SO_2 fumigation and acid dipping) have commercially been employed to control pericarp discolouration and decay in litchi fruit. However, the SO_2 and acid residue in commercial fruit possibly affects the sensory quality of fruit aril tissue, resulting in an off-flavour and taste (De Reuke *et al*., 2009). The taste effects from both SO_2 and acid surplus are starting to lead to a greater demand for non-adulterated litchi fruit in the market. Additionally, SO_2 treatment has been prohibited by many destination countries including the EU, USA and Japan (Holcroft *et al*., 2005) due to consumer health concerns. Therefore, alternative techniques for controlling postharvest quality without side-effects are preferred in response to concerns over food safety.

Although previous work has documented the effects of postharvest storage and handling on quality of stored litchi fruit, the reports mostly considered local fruit which usually was approx. 1 day old after harvest at the first day of experiment. As mentioned earlier, fresh litchi fruit has been supplied to overseas destination at least at levels of 500,000 tonnes annually. Thus, it is important to determine the appropriate conditions for maintaining postharvest quality of litchi fruit after arrival at overseas markets. The present study, therefore, aimed to detail the effects of different storage conditions and packaging materials on physiological and biochemical changes in aril and pericarp tissues of imported litchi fruit.

Management of temperature, relative humidity (RH) and vapour pressure deficit (VPD) during the supply chain are some of the most successful techniques for prolonging

postharvest life of fresh horticultural commodities. Optimum temperature, RH and VPD levels for each crop depend on several factors including fruit cultivar, postharvest handling and time. Appropriate temperature, RH and VPD not only delay the growth rate and spread of pathogens but also decelerate the rate of metabolic processes in fresh commodities. However, previous studies have mainly considered the effects of temperature and/or RH on litchi fruit pericarp discolouration (Tongdee *et al*., 1982; Jiang and Fu, 1999; Kaewchana *et al*., 2006). It would be of benefit for the consumer as well as the fruit industry to understand the alteration of quality-related compounds including sugar, organic acid, phenol and anthocyanin under storage conditions. The changes in physiological characteristics and biochemical composition during storage time in litchi aril and pericarp tissue were reported in this thesis. The effect of temperature, RH, VPD and modified atmosphere packaging (MAP) on respiration rate, CO_2 and ethylene production and accumulations, weight loss, and pericarp colour of fruit over storage time were all described. Changes in physiology were measured in conjunction with allied biochemical characteristics. However, the influences of temperature, RH, VPD, MAP and chemical treatment on litchi fruit physiology and biochemistry are summarised in Figure 8.1.

8.1.1. Physiological alteration

Results in Chapter 4 were the first to fully detail of the alterations of individual sugar, organic acid and total phenols in aril and individual anthocyanin and total phenols in pericarp tissue as well as physiological changes under different storage temperatures in 3 different crop seasons. A base study was conducted where imported fruit was stored at 5 different temperatures *viz*. 5, 8, 10, 13 and 20°C for 13 days. Predictably, temperature significantly affected fruit weight loss, whereby weight loss of fruit stored for 13 days at 5°C was lower than at 8, 10, 13 and 20°C (Figures 4.1 and 4.2). Similarly, storage at a low temperature (5°C) was reported to reduce weight loss in litchi fruit cvs. Hei Ye (Huang and Wang, 1990) and Wai Chee (Jacobi *et al*., 1993). Fruit kept at 5°C had a lower respiration rate, brighter red pericarp, higher concentrations of sugar, organic acids and total phenolics in both aril and pericarp tissue, and higher anthocyanin concentrations in pericarp tissue. The results from Chapter 4 were used to inform the design of the following

experiments, which were planned to study the alterations in physiology and biochemistry of imported litchi fruit held under different vapour pressure deficits (Chapter 5).

In Chapter 5, a new system using different glycerol solutions was employed to humidify the RH level (Figure 3.1). Although the effect of RH on postharvest changes in litchi fruit have been described in previous works (Jiang and Fu, 1999; Joas *et al*., 2005; Kaewchana et al., 2006), the recent study is the first report to detail the effects of VPD on litchi postharvest alterations. Temperature, RH and VPD were shown to significantly affect fruit weight loss and respiration rate (Figures 5.2 and 5.3, respectively) during 9 days storage. Weight loss of fruit from all storage regimes increased with storage time. Predictably, fruit stored at a lower RH lost significantly more weight than those fruit stored at a higher RH, which was in agreement with litchi cv. Hong Huay stored at 40-50 %RH (Kaewchana *et al*., 2006). Higher temperature increases the free energy of water molecules which, in turn, increases water movement and the potential for exchange with the atmosphere around the fruit (Kays and Paull, 2004) resulting in faster evapouration. At higher temperatures more moisture is required to saturate the air. A greater difference in vapour pressure (0.394 kPa) between the fruit and the storage atmosphere therefore leads to more rapid moisture loss from fruit to the environment (Figure 5.2). Hence, higher RH, lower temperature and/or lower VPD can minimise moisture loss in litchi fruit during storage.

Lower RH, higher temperature and/or higher VPD encouraged a greater respiratory rate during storage time. Respiration rate was negatively correlated with fruit weight loss. Fruit weight loss or dehydration could lead to pericarp browning, which possibly influenced the respiration rate. Chen *et al*. (1987) and Zhang and Quantick (2000) reported that respiration rate of stored litchi fruit also was affected by pericarp browning. Browning was mainly caused by dehydration of the pericarp. Litchi fruit stored at higher RH (> 90 %RH), lower temperature (5°C) and lower VPD (0.000-0.084 kPa) were brighter (higher L*) and were more red in colour (lower h°) than the pericarp of fruit held at lower RH and higher temperature (higher VPD), which was inconsistent with the study by Kaewchana *et al*. (2006) who found an increase in h° (*ca*. 220-225) of stored litchi cv. Hong Huay held at 20°C and 50-90 %RH. This could be due to the differences of fruit cultivar, growing region, postharvest handling and storage time or more probably the more precise control over RH% using in this thesis.

The results from Chapters 4 and 5 suggest that low temperature (5°C), high humidity (> 90 %RH) and low VPD (< 0.084 kPa) can minimise postharvest losses in stored litchi fruit. Controlled atmosphere storage (CA) or MAP could be a technique providing these atmosphere conditions. The effect of CA and MAP have been widely described to extend postharvest life of different fresh produce types e.g. citrus (Murata, 1997), banana (John and Marchal, 1995), pineapple (Paull and Rohrbach, 1985) and indeed litchi (Tongdee *et al*., 1982; Mahajan and Goswami, 2004) by decreasing metabolic rate and accordingly suppressing fruit ripening and senescence, and inhibiting pathogen growth (Thompson, 1998; Wills *et al*., 2005). In the current work, the effect of MAP on postharvest quality in stored litchi fruit was tested and discussed (Chapters 6 and 7). The influence of four different packaging films on physiology and biochemistry of non-adulterated litchi fruit have been explained in Chapter 6 whilst the effect of two types of plastic films on postharvest quality in non-adulterated and commercial (SO_2 and citric acid treated) fruit were detailed in Chapter 7.

The influence of MAP on fruit weight loss and CO_2 and ethylene production was described in Chapter 6 and 7. In Chapter 6, unwrapped fruit lost more weight than fruit packed with micro-perforated polypropylene, PropaFresh™ PFAM, Nature Flex™ NVS or Cellophane™ WS. Fruit weight loss, pericarp moisture content and dry matter in all treatments changed depending on the gas permeability of the plastic film used (Table 6.3). The influence of the gas permeability properties of plastic film on postharvest changes and quality of stored litchi fruit have been reported in previous studies (Table 6.1). PropaFresh™ PFAM film (H_2O and O_2 permeability: 3.173×10^{-17} and 7.521×10^{-18} $mol.s^{-1}.m.m^{-2}.Pa^{-1}$, respectively; Innovia Films) resulted in the least weight loss and thus maintained higher moisture contents in both aril and pericarp tissue, whilst greater weight loss and lower moisture contents were found in Nature Flex™ NVS packaged fruit (H_2O and O_2 permeability: 2.285×10^{-15} and 1.410×10^{-20} $mol.s^{-1}.m.m^{-2}.Pa^{-1}$, respectively; Table 6.2).

In Chapter 7, fruit stored at 5°C had lower fruit weight loss and higher pericarp moisture content than those kept at 13°C which was consistent with the results presented in Chapter 4 and 5. Although fruit weight loss was significantly higher in non-adulterated fruit, packaging films played a more important role in minimising weight loss of stored litchi fruit than chemical treatment (section 7.4.1). This could result from a difference in

the VP between the fruit and the storage atmosphere. Vapour pressure deficit between unwrapped fruit and the storage environment was apparently higher than the VPD between packaged fruit and the atmosphere inside the packages. Higher VPD accelerates moisture loss from fruit to the environment leading to higher weight loss, increase of pericarp dehydration and development of pericarp discolouration (Kays and Paull, 2004). It was therefore recommended that storage conditions for litchi should not only focus on maintenance of the cool chain, but should consider controlling the low VPD (< 0.068 kPa) to attain improved conservation of visual appearance.

Litchi is classified as a non-climacteric fruit, which produces much lower CO_2 and ethylene concentration (Wills *et al*., 2005). However, high concentrations of both CO_2 (*ca*. 3-28 kPa; Figure 6.1A and 7.1A) and ethylene (*ca*. 0.2-4.2 μL L^{-1}; Figure 6.1B and 7.1B) were detected in litchi packages. The high concentrations of CO_2 in Cellophane™ WS and NatureFlex™ NVS film regimes (Chapter 6), and moderate level of CO_2 in PropaFresh™ PFAM (Chapter 6 and 7) could be due mainly to their low gaseous transmission properties (Table 6.3) associated with fruit respiratory processes (CO_2 production). The elevated CO_2 level that accumulated in Cellophane™ WS and NatureFlex™ NVS treatments (after day 4) could act as a putative ethylene inhibitor (Burg and Burg, 1967) by repressing the production and activities of essential enzymes and substrates such as 1-aminocyclopropane-1-carboxlic acid (ACC), ACC synthase (ACS) and ACC oxidase (ACO) in the ethylene biosynthesis pathway (Kubo *et al*., 1996), whilst the moderate CO_2 concentration (*ca*. 2-3 kPa in PropaFresh™ PFAM over 9 days and in Cellophane™ WS and NatureFlex™ NVS during first 4 days) could enhance ethylene accumulation by promoting ACC, ACS and ACO (Pretel *et al*., 1999). Furthermore, high CO_2 concentrations within MAP can contribute to accumulation of acetaldehyde and ethanol thus leading to off-flavours in aril tissue (Pesis *et al*., 2002; Sivakumar and Korsten, 2006b). High CO_2 levels combined with water vapour in the packages may result in the production of carbonic acid and a subsequent decrease in pH, thereby inhibiting microbial growth and decay in packed fruit. This could have discouraged pathogen growth on fruit in Chapters 6 and 7 (see Appendix B.). Non-adulterated fruit in all packages produced higher CO_2 and ethylene concentrations than commercial fruit (Chapter 7). After chemical (i.e. SO_2 and acid) application, SO_2 and acid impregnation possibly provided a protective layer

on the surface of the pericarp against atmospheric oxygen which decelerated respiration rate and led to low CO_2 production and accumulation in the packages.

The results from previous chapters might suggest that ethylene apparently has an influence on postharvest deterioration in stored litchi fruit. The removal of ethylene and/or inhibition of effect of ethylene in storage has been reported to maintain the quality of mainly climacteric fresh produce (Saltveit, 1999). Although 1-methylcyclopropene (1-MCP) has been recently employed as ethylene inhibitor (Watkins, 2006), a novel palladium-promoted ethylene blocker has been developed to control ethylene induced ripening and senescence for several fruit (Terry *et al*., 2007b). The aforementioned ethylene inhibitors were employed to stored litchi fruit in the current work (mini trial) according to Terry *et al*. (2007c) with modification. Pd (3.5 g/ 12 fruit/ 13L chamber) and 1-MCP (10 μL L^{-1}/ 12 fruit/ 13L chamber) were applied to litchi fruit before being stored at 13°C for 11 days. The exogenous and endogenous ethylene content were minimised significantly with Pd, followed by 1-MCP and control regimes, respectively. Lower ethylene level in Pd chambers resulted in brighter colour (high L*) and more red colour (low h°) pericarp and less disease after 11 days as compared against 1-MCP and control treatments (Appendix C). The results from this trial indicated that Pd and 1-MCP have potential to prolong shelf life of litchi fruit. The effects of 1-MCP were documented to maintain postharvest quality in harvested litchi fruit by decreasing membrane integrity, pericarp browning, PPO and POD activities, respiration rate and retaining level of soluble solid concentration (SSC): titratable acidity (TA) and firmness during 21 days storage (Pang *et al*., 2001; De Reuck *et al*., 2009). However, there is a lack information on effect of ethylene scavengers on postharvest quality in stored litchi fruit. Future research, hence, could further explain the optimum concentration, timing of application, and format to prolong postharvest life of litchi fruit during long-term distribution.

Non-adulterated litchi fruit wrapped with PropaFresh™ PFAM film (Chapters 6 and 7) had a brighter red colour than those wrapped with micro-perforated polypropylene, Nature Flex™ NVS, Cellophane™ WS or unwrapped fruit. The high RH and moderate CO_2 and ethylene content in PropaFresh™ PFAM packages possibly minimised fruit and pericarp moisture loss, thus resulting in a brighter pericarp colour. However, fruit weight and pericarp moisture content did not influence the colour of acid treated fruit in Chapter 7. This could be due to the low pH in pericarp tissue of commercail fruit (not measured in

current work) which was sufficient to inhibit the anthocyanin and phenolic enzymatic epimerisation and degradation (Joas *et al*., 2005). Hence, combination of low temperature, high RH, low VPD, PropaFresh™ PFAM and chemical treatment reduced respiration rate, weight loss and pericarp discolouration in litchi fruit during storage.

8.1.2. Biochemical alteration

Temperature and/or RH did not influence total soluble solids (TSS) in litchi fruit in this research (Chapters 4, 5 and 7). However, unwrapped fruit (Chapters 6 and 7) contained higher TSS than those fruit wrapped with PropaFresh™ PFAM, NatureFlex™ NVS, Cellophane™ WS and micro-perforated polypropylene films. This result could be caused by the ambient CO_2 level in the unwrapped treatment leading to faster senescence than other film regimes. Higher moisture loss in unwrapped fruit also possibly increased the soluble solids content in aril tissue resulting in elevated TSS. In Chapter 7, TSS in commercial fruit was higher than in non-adulterated litchi. Although the largest proportion of soluble solids in litchi aril is made up of sugars (approx. 15% of edible portion; USDA, 2009), there was no correlation between TSS content and sugar concentrations (sucrose, glucose, fructose, total sugar or calculated sweetness) in aril tissue in all experiments (Chapters 4-7). This could be due to the narrow changes in refractometric level which was consistent with Batten (1989). Therefore, TSS, is not a suitable predictor of litchi sugar content.

Sucrose, glucose and fructose were the major sugars in litchi aril tissue. Fruit stored at high temperature, low RH and high VPD (Chapters 4 and 5) or unwrapped fruit (Chapters 6 and 7) or commercial fruit (Chapter 7) contained lower sucrose with higher glucose and fructose concentration than those fruit held at low temperature, high RH and low VPD or wrapped with plastic film or non-adulterated fruit. These results could be due to differences in fruit weight (moisture) loss leading to the hydrolysis of sucrose to form fructose and glucose together with the probable increase in sugar invertase enzyme activity during storage (Chan *et al*., 1975). The major sugars in litchi pericarp were glucose, mannose and fructose with minimal concentrations of sucrose. This is the first study to report the presence and abundance of these non-structural carbohydrates in litchi pericarp. High moisture content in pericarp could partially accelerate sucrose inversion, by the

action of acid or invertase enzyme, to form glucose and fructose (Shallenberger, 1993) resulting in trace levels of sucrose. Fruit stored at high RH, therefore, contained elevated glucose and fructose concentration in pericarp compared with fruit stored at lower RH. However, glucose decreased during 9 days storage whilst mannose and fructose increased (Chapter 6). The decrease in glucose concentration could be explained by the transformation of glucose to fructose and mannose structure in glycolysis and anthocyanin glycoside production or in the case of Lobry de Bruyn-Alberda van Ekenstein epimerisation (Angyal, 2001) during storage. However, SO_2 in fruit pericarp (Chapters 4-5) were possibly hydrolysed to sulphurous acid (Bridle and Timberlake, 1997) which led to inappropriate conditions for glucose-mannose-fructose transformation and resulted in high glucose concentration.

Lower temperature, higher RH, lower VPD and PropaFresh™ PFAM maintained higher organic acid concentrations in aril and pericarp tissue during storage time (9-13 days). Organic acids are a major source of energy for general metabolism, including the respiratory tricarboxylic acid cycle, in harvested produce. Lower organic acid concentrations recorded at higher storage temperature, lower RH and lower VPD could be due in part to the lower respiratory metabolism rate. Besides, high temperature and low RH storage resulted in an increase in SO_2 movement and absorption in litchi aril (Lemmer *et al*., 2000) which could result in off-flavour (not measured) in aril tissue which could have an impact on consumer acceptance, health and safety. Alterations in organic acid concentrations in the pericarp apparently influence pH, and therefore possibly sugar transformation and anthocyanin rutino/glucosides in the litchi pericarp. Endogenous organic acids and SO_2 residue in the pericarp can inhibit enzymatic browning and decelerate the decrease in organic acids in pericarp tissue. Therefore, the pericarp of commercial fruit in Chapter 7 retained higher acid concentrations than the pericarp of non-adulterated fruit during 11 days storage.

The lowest concentration of total phenolic compounds in aril and pericarp and individual anthocyanins in pericarp were found in litchis stored at high temperature (Chapters 4 and 5), low RH and VPD (Chapter 5) and without MAP (Chapters 6 and 7) during short storage (9-13 days). These treatments mainly resulted in fruit dehydration which could disrupt cellular compartmentalisation leading to an increase in enzymatic activities. The reduction in phenolic compounds in the aforementioned treatments might be

accelerated by enzymatic activities of polyphenol oxidase (PPO) and peroxidase (POD; Huang *et al*., 1990) which can contribute to browning of litchi pericarp. Anthocyanins can be transformed by anthocyanin-β-glucosidase (anthocyanase) (Jiang *et al*., 2004; Jiang *et al*., 2006) resulting in anthocyanidin and a sugar moiety, which then can be degraded by PPO and POD. However, SO_2 and acid treatment (Chapter 7) can prevent these enzymatic browning reactions by being hydrolysed to colourless chromen-2 (or chromen-4) sulphonic acid (quinine-sulphite complex) which has a similar structure and property to the carbitol form of the anthocyanin (Jurd, 1964; Bridle and Timberlake, 1997). There was no correlation between pericarp discolouration and anthocyanin deterioration in this study. This could be partly explained by the theory that visible pericarp discolouration is closely related to senescence, i.e. induced anthocyanin transformation (Figure 2.4) rather than degradation (Underhill *et al*., 1992). Besides, the lack of correlation between fruit colour and colour pigments may be an artifact of the objective measurement system used, since it may have been unable to account for the heterogeneity in colouration of non-adulterated fruit. It is likely that changes in pH will have altered the stability, co-pigmentation and spectra of the anthocyanins found in litchi fruit during storage. As a result, and despite anthocyanins being responsible for red pigmentation, the relationship between anthocyanins and litchi pericarp colour is still not fully understood.

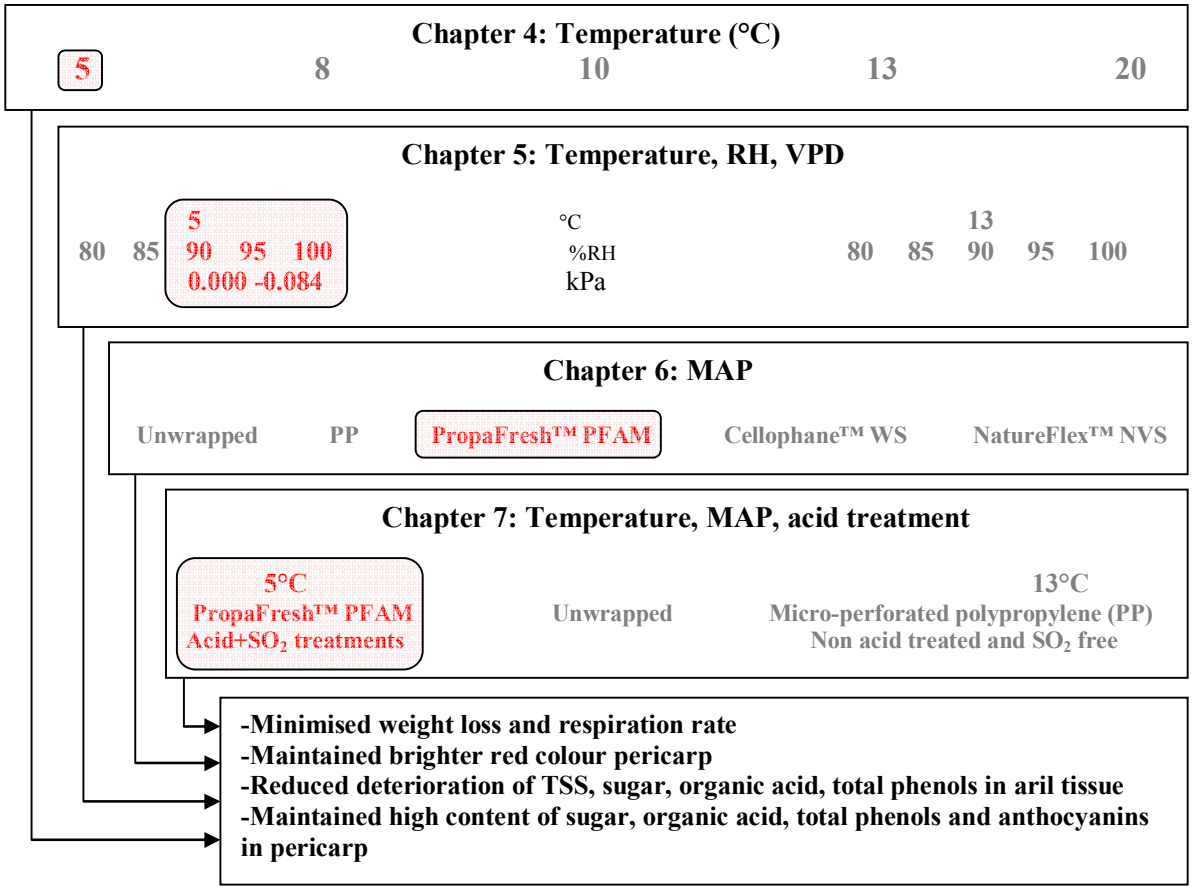


Figure 8.1. Summary of experimental work, results and conclusions. (Red label represented the best conditions for each chapter)

Fruit treated with PropaFresh™ PFAM maintained higher anthocyanin concentrations in fruit pericarp over storage time. Jiang and Fu (1999) documented that pH of litchi pericarp tissue was initially low but increased with pericarp desiccation. Possibly, high moisture content in the PropaFresh™ PFAM packaged fruit led to lower cellular pH in pericarp. Highly acidic conditions can stabilise the flavylium cation derivatives (anthocyanidin and their substitutions) and anthocyanin structure in litchi by decelerating the activity of PPO (Liu *et al*., 2007) and thus retain anthocyanin concentrations during storage. By contrast, the degradation of anthocyanins in unwrapped, micro-perforated polypropylene, Cellophane™ WS and NatureFlex™ NVS wrapped fruit could be a results of disruption of cellular compartmentalisation caused by moisture loss. This disruption allows PPO to react with phenolic substrates and results in the production of melanin

(brown pigments) in litchi pericarp (Underhill and Critchley, 1995). However, anthocyanin concentrations in the current study were not directly related to L*, C* and h° values. Additionally, the decrease in anthocyanins in Cellophane™ WS and NatureFlex™ NVS wrapped fruit may have been affected by elevated CO_2 and ethylene levels. Ethylene has been reported to enhance anthocyanin synthesis by increasing phenylalanine ammonia-lyase (PAL) activity. However, the high CO_2 level possibly inhibited ethylene production in Cellophane™ WS and NatureFlex™ NVS treatments (Figure. 6.1) which in turn might have reduced PAL activity and eventually anthocyanin concentration (Gil *et al*., 1997; Holcroft and Kader, 1999).

Disease was observed in fruit tested in Chapters 4 and 5, whilst no disease was recorded in Chapters 6 and 7. Pathogen growth in fruit was increased at higher storage temperature (13 and 20°C) and higher RH (95 and 100 %RH). Fruit kept at 13 and 20°C in (Chapter 4) had *ca.* 10 and 20%, respectively, disease coverage on the fruit surface after 13 days storage, whilst fruit held at 13°C with 95-100 %RH had *ca.* 5% disease coverage on the fruit surface after 9 days (Chapter 5). Although fruit tested in Chapter 7 did not show the disease symptoms during 11 days storage, pathogen growth was recorded in both commercial and non-adulterated fruit held at 13°C for 30 days. Major pathogen in commercial fruit were *Pennicilium* spp. (Sivakumar and Korsten. 2006) whilst a wide range of pathogens were detected in non-adulterated fruit (Appendix D.). However, this thesis does not aim to identify the changes in pathology in litchi fruit postharvest treatment, storage time or conditions.

Principal component analysis (PCA) is a non supervised multivariant technique and was employed in Chapter 5 to understand the influence of storage condition on physiological and biochemical changes in litchi fruit. PCA of litchi fruit (Figure 5.8) clearly demonstrated the clustering of the samples on PC 1 and PC 2 (68 and 16 % of the variance, respectively). Kom cultivar fruit were arranged away from cv. Mauritius fruit along PC 1 indicating a different reaction of each cultivar. Fruit cv. Mauritius kept at 5°C and 95-100 % RH (VPD = 0.000-0.084 kPa) were separated from those held at 13°C and 80-90 % RH (VPD = 0.137-0.274 kPa) treatments, respectively along PC 1. Although cv. Kom fruit sample could not be differentiated along PC 1, the samples were grouped separately into 5°C+80-85 % RH (VPD = 0.126-0.168 kPa), 5°C+90-100 % RH (VPD = 0.000-0.084 kPa), 13°C+80-85 % RH (VPD = 0.205-0.274 kPa) and 13°C+90-100 % RH

(VPD = 0.000-0.137 kPa) on PC 2. Respiration rate played the most important role in sample separation along PC 1 whilst aril glucose concentration was a key variable for PC 2. It is clear, therefore, that RH, temperature and VPD affected not only senescence but also carbohydrate utilization through respiration.

8.2. Recommendations for future experimental work

Pericarp discolouration and dehydration are the major causes of postharvest loss in litchi fruit. Further research on the influences of storage conditions e.g. vapour pressure deficit control, especially under commercial conditions would be of interest, despite it is difficult to design an experiment due to the number of factors involved. The use of organoleptic analysis to assess aril quality after storage would also be of valuable in completely defining the effects of the imposed storage conditions.

Most fruit used for this study were fumigated with SO_2. The effect of SO_2 on anthocyanin stability is widely discussed, but no analysis of SO_2 content in the pericarp was undertaken. The relationship between SO_2, anthocyanin and pericarp browning could be important, for example the activity of PPO depends on the presence of SO_2, and so it would be useful to measure the rate of SO_2 degradation in pericarp and aril tissue.

A wide variety of packaging materials have previously been studied to minimise postharvest loss in litchi fruit (Table 6.1). Although Propafresh™ PFAM was the best film of those tested in this thesis to maintain quality of stored litchi, it is classified as non-biodegradable. Use of non-biodegradable material leads to increased waste and environmental pollution. The Cellophane™ WS and NatureFlex™ NVS films used in the current study were biodegradable but did not sufficiently maintain postharvest quality of litchi fruit during 9 days storage (Chapter 6). The plastic film industry has developed bio-degradable base films for use in the cool chain, but the most recently developed bio-degradable films are for non to low perishable products. Hence, the effects of bio-degradable film for prolonging postharvest life in litchi fruit would be recommended for further research.

In addition, postharvest loss found in Chapters 4-7 apparently affected litchi fruit in aesthetic appeal (e.g. pericarp colour) rather than in taste-related compound (e.g. sugar and organic acid). These results could change consumer opinions by increasing understanding

and appreciation that a litchi fruit with poor fruit visual appearance does not necessary mean that it will have lesser eating quality.

8.3. Project conclusions

The project objectives were set out in Chapter 1, Section 1.2.2. A brief summary of the conclusions of the project in terms of the objectives is detailed below.

- *Determine the postharvest temporal and spatial changes in litchi as affected by temperature, relative humidity (RH), vapour pressure deficit (VPD), gaseous regimes and packaging films.* Temperature, RH, VPD and MAP affected postharvest quality of litchi fruit during short term storage (9-13 days). Low temperature (Chapter 4), high RH, low VPD (Chapters 4 and 5) and MAP (Chapters 6 and 7) generally decelerated respiration rate and weight loss which in turn maintained red colour in fruit pericarp during storage. These storage conditions also maintained higher concentrations of total soluble solids, sugars (sucrose, glucose, fructose and mannose (mannose found only in pericarp tissue); Chapters 4-7), organic acids (malic, tartaric, citric, ascorbic and oxalic acids; Chapters 4-7), and total phenols (Chapter 4) in fruit aril and pericarp tissue and anthocyanins in pericarp tissue (cyanidin 3-rutinoside, cyanidin 3-glucoside and malvidin 3-glcoside; Chapters 4-7) during storage.

- *Identify the optimum conditions for maintenance of litchi quality using chemometric analysis obtained from objective 1.* Storage at 5°C with ≥ 95 % RH and VPD < 0.000-0.084 (Chapters 4 and 5) are the optimum storage conditions to maintain postharvest quality of litchi fruit.

- *Select appropriate packaging to achieve optimum conditions.* PropaFresh™ PFAM is the best plastic film of those tested for storing litchi fruit during 9-11 days storage (Chapters 6 and 7) whereby it maintained postharvest quality of litchi fruit better than NatureFlex™ NVS, Cellophane™ WS, micro-perforated polypropylene or

unwrapped fruit by minimising weight loss and CO_2 and ethylene accumulation, and maintaining concentrations of sugars, organic acids, phenols and anthocyanins.

CHAPTER NINE

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APPENDIX A.

STATISTIC TABLES

ANOVA tables

Table A1. Respiration rate of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	328.08	65.62	15.65	<.001
Storage temperature	3	1617.64	539.21	128.64	<.001
Storage time. Storage temperature	15	1189.47	79.30	18.92	<.001
Residual	48	201.19	4.19		
Total	71	3336.37			

Table A2. Respiration rate of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	2005.00	401.00	58.77	<.001
Storage temperature	2	2172.91	1086.45	159.24	<.001
Storage time. Storage temperature	7 (3)	891.81	127.40	18.67	<.001
Residual	30 (6)	204.68	6.82		
Total	44 (9)	4483.71			

Table A3. Respiration rate of Experiment 3

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage time	5	3073.78	614.76	34.63	<.001
Storage temperature	2	6838.24	3419.12	192.62	<.001
Storage time. Storage temperature	8 (2)	3217.36	402.17	22.66	<.001
Residual	32 (4)	568.01	17.75		
Total	47 (6)	11420.76			

Table A4. Weight loss (%) of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	474.08	94.81	224.22	<.001
Storage temperature	3	40.84	13.61	32.20	<.001
Storage time. Storage temperature	15	16.80	1.12	2.65	<.001
Residual	408	172.53	0.42		
Total	431	704.27			

Table A5. Weight loss (%) of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	809057	161811	70.93	<.001
Storage temperature	2	1763290	881645	386.49	<.001
Storage time. Storage temperature	10	658645	65865	28.87	<.001
Residual	306	698028	2281		
Total	323	3929020			

Table A6. Weight loss (%) of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1920.09	384.019	86.53	<.001
Storage temperature	2	5398.54	2699.27	608.21	<.001
Storage time. Storage temperature	10	2084.59	208.46	46.97	<.001
Residual	306	1358.04	4.43		
Total	323	10761.28			

Table A7. Aril moisture content of litchi fruit of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	20.34	5.08	3.06	0.170
Storage time	5	24.11	6.03	3.63	0.060
Temperature. Storage time	14	46.59	2.91	1.75	0.235
Residual	425	705.28	1.66		
Total	431	796.31			

Table A8. Aril moisture content of litchi fruit of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	18.34	9.17	5.56	0.108
Storage time	5	20.13	4.03	2.44	0.221
Temperature. Storage time	9	44.00	4.89	2.96	0.314
Residual	306	505.76	1.65		
Total	323	598.24			

Table A9. Aril moisture content of litchi fruit of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	24.77	12.39	8.32	0.370
Storage time	5	20.41	4.08	2.74	0.120
Temperature. Storage time	9	50.12	5.57	3.74	0.096
Residual	306	458.39	1.49		
Total	323	568.26			

Table A10. Pericarp moisture content of litchi fruit of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	80.34	26.78	22.50	0.032
Storage time	5	84.11	16.82	14.13	0.010
Temperature. Storage time	14	106.59	7.61	6.39	0.005
Residual	425	505.28	1.19		
Total	431	600.31			

Table A11. Pericarp moisture content of litchi fruit of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	58.38	29.19	25.16	<.001
Storage time	5	55.13	11.03	9.51	0.035
Temperature. Storage time	9	94.00	10.44	9.00	0.004
Residual	306	355.76	1.16		
Total	323	408.25			

Table A12. Pericarp moisture content of litchi fruit of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	25.73	12.87	8.64	0.001
Storage time	5	27.41	5.48	3.68	0.003
Temperature. Storage time	9	46.12	5.12	3.44	<.001
Residual	306	442.30	1.49		
Total	323	507.26			

Table A13. Aril dry matter of litchi fruit of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	20.34	5.08	3.06	0.170
Storage time	5	24.11	6.03	3.63	0.060
Temperature. Storage time	14	46.59	2.91	1.75	0.235
Residual	425	705.28	1.66		
Total	431	796.31			

Table A8. Aril dry matter of litchi fruit of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	18.34	9.17	5.56	0.108
Storage time	5	20.13	4.03	2.44	0.221
Temperature. Storage time	9	44.00	4.89	2.96	0.314
Residual	306	505.76	1.65		
Total	323	598.24			

Table A9. Aril dry matter of litchi fruit of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	24.77	12.39	8.32	0.370
Storage time	5	20.41	4.08	2.74	0.120
Temperature. Storage time	9	50.12	5.57	3.74	0.096
Residual	306	458.39	1.49		
Total	323	568.26			

Table A10. Pericarp dry matter of litchi fruit of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	3	80.34	26.78	22.50	0.002
Storage time	5	84.11	16.82	14.13	0.001
Temperature. Storage time	14	106.59	7.61	6.39	0.006
Residual	425	505.28	1.19		
Total	431	600.31			

Table A11. Pericarp dry matter of litchi fruit of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	58.38	29.19	25.16	<.001
Storage time	5	55.13	11.03	9.51	0.015
Temperature. Storage time	9	94.00	10.44	9.00	0.008
Residual	306	355.76	1.16		
Total	323	408.25			

Table A12. Pericarp dry matter of litchi fruit of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	2	25.73	12.87	8.64	0.001
Storage time	5	27.41	5.48	3.68	0.003
Temperature. Storage time	9	46.12	5.12	3.44	<.001
Residual	306	442.30	1.49		
Total	323	507.26			

Table A13. L^* of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1408.04	281.61	10.82	<.001
Storage temperature	3	267.32	89.11	3.42	0.017
Storage time. Storage temperature	15	634.33	42.29	1.62	0.064
Residual	408	10620.84	26.03		
Total	431	1290.53			

Table A14. *L**of Experiment 2

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage time	5	4808.57	961.71	54.23	<.001
Storage temperature	2	2925.28	1462.64	82.48	<.001
Storage time. Storage temperature	10	788.53	78.85	4.45	<.001
Residual	305 (1)	5408.62	17.73		
Total	322 (1)	13859.79			

Table A15. *L**of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	4206.19	841.24	32.24	<.001
Storage temperature	2	3330.63	1665.31	63.82	<.001
Storage time. Storage temperature	10	812.19	81.22	3.11	<.001
Residual	306	7985.06	26.09		
Total	323	16334.07			

Table A16. *C** of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	127.89	25.57	3.67	0.003
Storage temperature	3	29.43	9.81	4.41	0.239
Storage time. Storage temperature	15	155.66	10.37	1.49	0.105
Residual	408	2840.59	6.96		
Total	431	3153.58			

Table A17. *C** of Experiment 2

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage time	5	84.54	16.91	2.58	0.027
Storage temperature	2	22.63	11.32	1.72	0.180
Storage time. Storage temperature	10	127.63	12.76	1.95	0.039
Residual	305 (1)	2000.65	6.56		
Total	322 (1)	2234.69			

Table A18. *C** of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	7.04	1.408	0.25	0.941
Storage temperature	2	123.58	61.79	10.87	<.001
Storage time. Storage temperature	10	164.71	16.47	2.90	0.002
Residual	306	1739.86	5.69		
Total	323	2035.18			

Table A19. h° of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	5041.50	1008.3	4.37	<.001
Storage temperature	3	5667.70	1889.20	8.19	<.001
Storage time. Storage temperature	15	2574.20	171.60	0.74	0.740
Residual	408	94172.70	230.80		
Total	431	107456.20			

Table A20. h° of Experiment 2

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage time	5	11205.50	2241.10	19.43	<.001
Storage temperature	2	3113.70	1556.80	13.50	<.001
Storage time. Storage temperature	10	2124.20	212.40	1.84	0.053
Residual	305 (1)	35183.90	115.40		
Total	322 (1)	51614.50			

Table A21. h° of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	6317.80	1263.60	8.46	<.001
Storage temperature	2	3318.00	1659.00	11.11	<.001
Storage time. Storage temperature	10	2527.20	252.70	1.69	0.082
Residual	306	45712.20	149.40		
Total	323	57875.10			

Table A22. TSS of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	108.67	21.73	22.47	<.001
Storage temperature	3	30.43	10.14	10.49	<.001
Storage time. Storage temperature	15	19.12	1.27	1.32	0.187
Residual	408	394.65	0.97		
Total	431	552.88			

Table A23. TSS of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	2.73	0.55	0.74	0.596
Storage temperature	2	17.50	8.75	11.83	<.001
Storage time. Storage temperature	10	34.57	3.46	4.67	<.001
Residual	306	226.38	0.74		
Total	323	281.18			

Table A24. TSS of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	9.14	1.83	0.99	0.425
Storage temperature	2	50.13	25.06	13.54	<.001
Storage time. Storage temperature	10	100.93	10.10	5.45	<.001
Residual	306	566.32	1.85		
Total	323	726.51			

Table A25. Aril sucrose concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	194040.00	38808	5.34	<.001
Storage temperature	3	202711.00	67570	9.30	<.001
Storage time. Storage temperature	15	146043.00	9736	1.34	0.175
Residual	408	2964720.00	7266		
Total	431	3507514.00			

Table A26. Aril sucrose concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	9836.5	1967.3	4.34	0.003
Storage temperature	2	47031.9	23515.9	51.85	<.001
Storage time. Storage temperature	10	7454.4	745.4	1.64	0.134
Residual	36	16328.1	453.6		
Total	53	80650.90			

Table A27. Aril sucrose concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	32461.50	6492.30	16.34	<.001
Storage temperature	2	4971.10	2485.60	6.26	0.005
Storage time. Storage temperature	10	1884.70	188.50	0.47	0.896
Residual	36	14303.3	397.30		
Total	53	53620.60			

Table A28. Aril glucose concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	3233.8	646.8	2.85	0.015
Storage temperature	3	6344.1	2114.7	9.33	<.001
Storage time. Storage temperature	15	10663.0	710.9	3.14	<.001
Residual	408	92501.5	226.7		
Total	431	112742.4			

Table A29. Aril glucose concentration of Experiment 2

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Storage time	5	1351.30	270.30	1.97	0.107
Storage temperature	2	22867.30	11433.70	83.27	<.001
Storage time. Storage temperature	10	1372.30	137.20	1.00	0.462
Residual	36	4942.90	137.30		
Total	53	30533.90			

Table A30. Aril glucose concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	23779.30	4755.90	20.47	<.001
Storage temperature	2	142.20	71.10	0.31	0.738
Storage time. Storage temperature	10	10225.60	10225.60	4.40	<.001
Residual	36	8362.50	8362.50		
Total	53	42509.50	42509.50		

Table A31. Aril fructose concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	38004.00	7601.00	3.17	0.008
Storage temperature	3	35154.00	11718.00	4.89	0.002
Storage time. Storage temperature	15	71896.00	4793.00	2.00	0.014
Residual	408	977783.00	2397.00		
Total	431	1122837.00			

Table A32. Aril fructose concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	10584.90	2117.00	7.61	<.001
Storage temperature	2	183881.00	91940.50	330.52	<.001
Storage time. Storage temperature	10	7622.20	762.20	2.74	0.013
Residual	36	10014.20	278.20		
Total	53	212102.30			

Table A33. Aril fructose concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	20767.60	4153.50	15.76	<.001
Storage temperature	2	304.60	152.30	0.58	0.566
Storage time. Storage temperature	10	8763.00	876.30	3.33	0.004
Residual	36	9486.20	263.50		
Total	53	39321.40			

Table A34. Ascorbic acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	309.95	61.99	13.51	<.001
Storage temperature	3	115.90	38.63	.42	<.001
Storage time. Storage temperature	15	263.06	17.54	3.82	<.001
Residual	408	1871.47	4.59		
Total	431	2560.38			

Table A35. Ascorbic acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	17.13	3.43	2.17	0.079
Storage temperature	2	19.25	9.63	6.11	0.005
Storage time. Storage temperature	10	18.18	1.82	1.15	0.352
Residual	36	56.70	1.58		
Total	53	111.25			

Table A36. Ascorbic acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	17.13	3.43	2.17	0.079
Storage temperature	2	19.25	9.63	6.11	0.005
Storage time. Storage temperature	10	18.18	1.82	1.15	0.352
Residual	36	56.70	1.58		
Total	53	111.25			

Table A37. Citric concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	148.56	29.71	6.31	<.001
Storage temperature	3	279.82	93.27	19.82	<.001
Storage time. Storage temperature	15	301.22	20.08	4.27	<.001
Residual	408	1920.28	4.71		
Total	431	2649.87			

Table A38. Citric concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	6.05	1.21	0.64	0.069
Storage temperature	2	2.15	1.07	0.57	0.071
Storage time. Storage temperature	10	10.86	1.09	0.58	0.022
Residual	36	67.87	1.89		
Total	53	86.93			

Table A39. Citric concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	17.13	3.43	2.17	0.049
Storage temperature	2	19.25	9.63	6.11	0.005
Storage time. Storage temperature	10	18.18	1.82	1.15	0.052
Residual	36	56.70	1.58		
Total	53	111.25			

Table A40. Malic acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	2474.90	495.00	3.83	0.002
Storage temperature	3	3176.30	1058.80	8.19	<.001
Storage time. Storage temperature	15	5131.70	342.10	2.65	<.001
Residual	408	52715.50	129.20		
Total	431	63498.50			

Table A41. Malic acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	272.93	54.59	5.07	0.001
Storage temperature	2	18.93	9.47	0.88	0.024
Storage time. Storage temperature	10	579.72	57.97	5.38	<.001
Residual	36	387.61	10.77		
Total	53	1259.20			

Table A42. Malic acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	17.13	3.43	2.17	0.039
Storage temperature	2	19.25	9.63	6.11	0.005
Storage time. Storage temperature	10	18.18	1.82	1.15	0.032
Residual	36	56.70	1.58		
Total	53	111.25			

Table A43. Oxalic acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	38.66	7.73	9.44	<.001
Storage temperature	3	45.94	15.31	18.70	<.001
Storage time. Storage temperature	15	42.09	2.81	3.43	<.001
Residual	408	334.14	0.82		
Total	431	460.83			

Table A44. Oxalic acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1.84	0.37	4.66	0.002
Storage temperature	2	8.40	4.20	53.07	<.001
Storage time. Storage temperature	10	2.84	0.28	3.58	0.002
Residual	36	2.85	0.08		
Total	53	15.93			

Table A45. Oxalic acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	17.13	3.43	2.17	0.049
Storage temperature	2	19.25	9.63	6.11	0.005
Storage time. Storage temperature	10	18.18	1.82	1.15	0.032
Residual	36	56.70	1.58		
Total	53	111.25			

Table A46. Tartaric acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	38.66	7.73	9.44	<.001
Storage temperature	3	45.94	15.31	18.70	<.001
Storage time. Storage temperature	15	42.09	2.81	3.43	<.001
Residual	408	334.14	0.82		
Total	431	460.83			

Table A47. Tartaric acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	5.76	1.15	0.98	0.045
Storage temperature	2	11.58	5.79	4.91	0.013
Storage time. Storage temperature	10	72.68	7.27	6.16	<.001
Residual	36	42.45	1.18		
Total	53	132.46			

Table A48. Tartaric acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	17.13	3.43	9.44	<.001
Storage temperature	2	19.25	9.63	18.70	<.001
Storage time. Storage temperature	10	18.18	1.82	3.43	<.001
Residual	36	56.70	1.58		
Total	53	111.25			

Table A49. Ascorbic acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	374.10	74.80	0.52	0.037
Storage temperature	3	2484.70	828.20	5.80	0.002
Storage time. Storage temperature	15	2635.10	175.70	1.23	0.023
Residual	408	6848.90	142.70		
Total	431	12342.80			

Table A50. Ascorbic acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	5421.80	1084.36	34.00	<.001
Storage temperature	2	4877.10	2438.55	76.46	<.001
Storage time. Storage temperature	10	10657.26	1065.73	33.41	<.001
Residual	36	1148.23	31.90		
Total	53	22104.39			

Table A51. Ascorbic acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	600.77	120.15	2.79	0.031
Storage temperature	2	89.83	44.92	1.04	0.033
Storage time. Storage temperature	10	861.92	86.19	2.00	0.042
Residual	36	1549.09	43.03		
Total	53	3101.61			

Table A52. Citric acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	6.69	1.34	0.81	0.046
Storage temperature	3	91.34	30.45	18.52	<.001
Storage time. Storage temperature	15	32.04	2.14	1.30	0.040
Residual	408	78.90	1.64		
Total	431	208.96			

Table A53. Citric acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	19.57	3.91	7.90	<.001
Storage temperature	2	1.16	0.58	1.17	0.022
Storage time. Storage temperature	10	55.27	5.53	11.15	<.001
Residual	36	17.84	0.50		
Total	53	93.84			

Table A54. Citric acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	22.47	4.49	3.00	0.023
Storage temperature	2	8.88	4.44	2.96	0.065
Storage time. Storage temperature	10	21.58	2.16	1.44	0.023
Residual	36	54.00	1.50		
Total	53	106.92			

Table A55. Malic acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	3.73	0.75	0.70	0.027
Storage temperature	3	20.64	6.88	6.44	<.001
Storage time. Storage temperature	15	36.26	2.42	2.26	0.017
Residual	408	51.26	1.07		
Total	431	111.88			

Table A56. Malic acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	45.39	9.08	37.50	<.001
Storage temperature	2	17.54	8.77	36.23	<.001
Storage time. Storage temperature	10	85.15	8.52	35.17	<.001
Residual	36	8.72	0.24		
Total	53	156.80			

Table A57. Malic acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	2.83	0.57	1.88	0.123
Storage temperature	2	3.29	1.65	5.46	0.008
Storage time. Storage temperature	10	9.15	0.92	3.04	0.007
Residual	36	10.85	0.30		
Total	53	26.12			

Table A58. Oxalic acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1.35	0.27	0.44	0.019
Storage temperature	3	5.49	1.83	2.98	0.041
Storage time. Storage temperature	15	16.63	1.11	1.80	0.042
Residual	408	29.52	0.62		
Total	431	53.00			

Table A59. Oxalic acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	46.90	9.38	2.74	0.034
Storage temperature	2	27.22	13.61	3.97	0.028
Storage time. Storage temperature	10	60.17	6.02	1.76	0.105
Residual	36	123.33	3.43		
Total	53	257.61			

Table A60. Oxalic acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	173.89	34.78	2.89	0.027
Storage temperature	2	29.06	14.53	1.21	0.311
Storage time. Storage temperature	10	144.22	14.42	1.20	0.325
Residual	36	433.80	12.05		
Total	53	780.97			

Table A61. Tartaric acid concentration of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	0.63	0.13	0.72	0.010
Storage temperature	3	2.57	0.86	7.93	0.005
Storage time. Storage temperature	15	5.93	0.40	2.28	0.016
Residual	408	8.33	0.17		
Total	431	17.45			

Table A62. Tartaric acid concentration of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	35.43	7.09	15.80	<.001
Storage temperature	2	16.98	8.49	18.92	<.001
Storage time. Storage temperature	10	68.82	6.88	15.34	<.001
Residual	36	16.15	0.45		
Total	53	137.37			

Table A63. Tartaric acid concentration of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	39.63	7.93	11.65	<.001
Storage temperature	2	12.48	6.24	9.18	<.001
Storage time. Storage temperature	10	18.47	1.85	2.72	0.014
Residual	36	24.48	0.68		
Total	53	95.06			

Table A64. Total phenolic contents of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1040.70	208.10	1.45	0.023
Storage temperature	3	1305.90	435.30	3.04	0.038
Storage time. Storage temperature	15	3931.00	262.10	1.83	0.048
Residual	48	6876.50	143.30		
Total	71	13154.10			

Table A65. Total phenolic contents of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1186.16	237.23	5.42	<.001
Storage temperature	2	85.91	42.95	0.98	0.004
Storage time. Storage temperature	10	527.53	52.75	1.21	0.020
Residual	36	1574.61	43.74		
Total	53	3374.21			

Table A66. Total phenolic contents of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	2195.20	439.00	1.06	0.007
Storage temperature	2	752.60	376.30	0.91	0.011
Storage time. Storage temperature	10	3308.30	330.80	0.80	0.029
Residual	36	14870.60	413.10		
Total	53	21126.70			

Table A67. Total phenolic contents in pericarp of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1040.70	208.10	1.45	0.023
Storage temperature	3	1305.90	435.30	3.04	0.038
Storage time. Storage temperature	15	3931.00	262.10	1.83	0.048
Residual	48	6876.50	143.30		
Total	71	13154.10			

Table A68. Total phenolic contents in pericarp of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	1186.16	237.23	5.42	<.001
Storage temperature	2	85.91	42.95	0.98	0.024
Storage time. Storage temperature	10	527.53	52.75	1.21	0.026
Residual	36	1574.61	43.74		
Total	53	3374.21			

Table A69. Total phenolic contents in pericarp of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	2195.20	439.00	1.06	0.017
Storage temperature	2	752.60	376.30	0.91	0.021
Storage time. Storage temperature	10	3308.30	330.80	0.80	0.029
Residual	36	14870.60	413.10		
Total	53	21126.70			

Table A70. Cyanidin 3-glucoside of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	208.87	41.77	1.22	0.016
Storage temperature	3	464.37	154.79	4.50	0.007
Storage time. Storage temperature	15	948.64	63.24	1.84	0.056
Residual	48	1649.71	34.37		
Total	71	3271.58			

Table A71. Cyanidin 3-glucoside of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	4382.14	876.43	11.71	<.001
Storage temperature	2	2267.12	1133.56	15.15	<.001
Storage time. Storage temperature	10	7538.81	753.88	10.08	<.001
Residual	36	2693.34	74.81		
Total	53	16881.41			

Table A72. Cyanidin 3-glucoside of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	787.80	157.60	1.30	0.017
Storage temperature	2	5483.60	2741.80	22.56	<.001
Storage time. Storage temperature	10	4432.60	443.30	3.65	0.002
Residual	36	4375.80	121.60		
Total	53	15079.80			

Table A73. Cyanidin 3-rutinoside of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	37099.00	7420.00	0.90	0.028
Storage temperature	3	91884.00	30628.00	3.72	0.017
Storage time. Storage temperature	15	158932.00	10595.00	1.29	0.047
Residual	48	395276.00	8235.00		
Total	71	683191.00			

Table A74. Cyanidin 3-rutinoside of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	951471.00	190294.00	10.19	<.001
Storage temperature	2	361740.00	180870.00	9.69	<.001
Storage time. Storage temperature	10	724564.00	72456.00	3.88	0.001
Residual	36	672281.00	18674.00		
Total	53	2710056.00			

Table A75. Cyanidin 3-rutinoside of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	382177.00	76435.00	2.34	0.032
Storage temperature	2	1359629.00	679814.00	20.77	<.001
Storage time. Storage temperature	10	1203892.00	120389.00	3.68	0.002
Residual	36	1178102.00	32725.00		
Total	53	4123799.00			

Table A76. Malvidin 3-glucoside of Experiment 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	95.30	19.06	3.66	0.007
Storage temperature	3	82.72	27.57	5.29	0.003
Storage time. Storage temperature	15	258.79	17.25	3.31	<.001
Residual	48	250.15	5.21		
Total	71	686.95			

Table A77. Malvidin 3-glucoside of Experiment 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	201.17	40.84	4.73	0.002
Storage temperature	2	26.45	13.22	1.53	0.030
Storage time. Storage temperature	10	114.52	11.45	1.33	0.024
Residual	36	310.72	8.63		
Total	53	655.86			

Table A78. Malvidin 3-glucoside of Experiment 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	5	86.64	17.33	3.14	0.019
Storage temperature	2	150.88	75.44	13.66	<.001
Storage time. Storage temperature	10	95.01	9.50	1.72	0.014
Residual	36	198.76	5.52		
Total	53	531.29			

Table A79. Respiration rate of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	169.72	42.43	8.80	<.001
Temperature	1	664.58	664.58	132.81	<.001
Storage time	4	1461.23	401.00	58.77	<.001
RH.Temperature	4	82.86	20.71	4.29	<.001
RH. Storage time	16	175.99	10.99	2.01	<.001
Temperature. Storage time	4	1408.41	352.10	73.06	<.001
RH. Temperature. Storage time.	20	891.00	14.92	21.89	<.001
Residual	40	204.74	5.00		
Total	53	5128.89			

Table A80. Respiration rate of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	130.77	32.69	39.62	<.001
Temperature	1	2414.23	2414.24	2926.00	<.001
Storage time	4	367.59	122.53	148.50	<.001
RH.Temperature	4	48.16	12.04	14.59	<.001
RH. Storage time	16	43.12	3.59	4.36	<.001
Temperature. Storage time	4	214.28	71.43	86.57	<.001
RH. Temperature. Storage time.	20	65.43	5.45	6.61	<.001
Residual	40	66.01	0.83		
Total	53	3349.60			

Table A81. Weight loss (%) of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	54.28	13.57	15.63	<.001
Temperature	1	18.03	18.03	20.77	<.001
Storage time	4	474.08	94.81	53.45	<.001
RH. Temperature	4	6.41	1.60	1.85	0.126
RH. Storage time	16	63.34	46.41	4.56	0.002
Temperature. Storage time	4	16.10	4.03	4.64	<.001
RH. Temperature. Storage time	16	11.26	13.61	0.81	0.671
Residual	100	86.82	0.70		
Total	299	441.90			

Table A82. Weight loss (%) of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	46.99	11.75	49.56	<.001
Temperature	1	19.00	19.01	80.18	<.001
Storage time	4	60.58	15.15	63.89	<.001
RH. Temperature	4	5.66	1.42	5.97	<.001
RH. Storage time	16	29.82	1.86	7.86	<.001
Temperature. Storage time	4	14.59	3.64	15.38	<.001
RH. Temperature. Storage time	16	10.73	0.67	2.83	<.001
Residual	100	23.71	0.24		
Total	299	211.08			

Table A83. Aril moisture content (%) of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	5.87	1.47	0.03	0.874
Temperature	1	0.04	0.04	0.91	0.461
Storage time	4	11.60	2.90	1.80	0.135
RH. Temperature	4	5.33	1.33	0.83	0.512
RH. Storage time	16	29.00	1.81	1.23	0.304
Temperature. Storage time	4	7.93	1.98	1.12	0.344
RH. Temperature. Storage time	16	33.46	2.09	1.30	0.214
Residual	100	161.27	1.61		
Total	299	254.50			

Table A84. Aril moisture content (%) of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	159.48	39.87	5.55	0.055
Temperature	1	10.17	10.17	1.42	0.237
Storage time	4	19.02	4.76	0.66	0.620
RH. Temperature	4	152.50	38.13	5.31	<.001
RH. Storage time	16	141.81	8.86	1.23	0.256
Temperature. Storage time	4	15.37	3.84	0.54	0.710
RH. Temperature. Storage time	16	83.74	5.23	0.73	0.759
Residual	100	718.09	7.18		
Total	299	1300.17			

Table A85. Pericarp moisture content (%) of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	3.38	3.38	0.05	0.824
Temperature	1	479.98	120.00	1.76	0.143
Storage time	4	225.06	56.27	0.82	0.513
RH. Temperature	4	175.08	43.77	0.64	0.635
RH. Storage time	16	335.83	83.96	1.23	0.303
Temperature. Storage time	4	2135.38	133.46	1.95	0.024
RH. Temperature. Storage time	16	799.60	49.97	0.73	0.756
Residual	100	6829.23	68.29		
Total	299	10983.54			

Table A86. Pericarp moisture content (%) of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	1789.10	447.3	1.20	0.031
Temperature	1	117.40	117.4	0.31	0.050
Storage time	4	3214.80	803.7	2.15	0.080
RH. Temperature	4	1429.00	357.3	0.96	0.040
RH. Storage time	16	5530.20	345.6	0.93	0.543
Temperature. Storage time	4	602.70	150.7	0.40	0.806
RH. Temperature. Storage time	16	2759.10	172.4	0.46	0.959
Residual	100	37331.40	373.3		
Total	299	52773.80			

Table A87. *L** of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	924.36	231.09	6.78	<.001
Temperature	1	135.24	135.24	3.97	0.049
Storage time	4	191.75	281.61	1.41	0.029
RH. Temperature	4	85.29	47.94	0.63	0.646
RH. Storage time	16	201.59	21.32	0.37	0.987
Temperature. Storage time	4	353.28	12.60	2.59	0.041
RH. Temperature. Storage time	16	559.23	88.32	1.02	0.438
Residual	100	3410.27	34.95		0.038
Total	299	5861.01	34.10		

Table A88. *L**of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	17.13	4.28	0.17	0.953
Temperature	1	75.19	75.19	2.98	0.088
Storage time	4	404.54	101.13	4.00	0.005
RH. Temperature	4	32.36	8.09	0.32	0.864
RH. Storage time	16	440.65	11.96	0.47	0.755
Temperature. Storage time	4	47.83	27.54	1.09	0.374
RH. Temperature. Storage time	16	602.28	37.64	1.49	0.118
Residual	100	2526.06	25.26		
Total	299	4146.04			

Table A89. *C** of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	5.94	1.49	0.31	0.087
Temperature	1	48.88	48.88	10.18	0.002
Storage time	4	9.82	2.46	0.51	0.703
RH. Temperature	4	29.32	7.33	1.53	0.200
RH. Storage time	16	48.87	3.06	0.64	0.847
Temperature. Storage time	4	22.85	5.71	1.19	0.320
RH. Temperature. Storage time	16	94.36	5.90	1.23	0.260
Residual	100	480.01	4.80		
Total	299	740.07			

Table A90. *C** of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	22.11	5.53	0.88	0.488
Temperature	1	3.03	3.03	0.48	0.476
Storage time	4	23.70	5.93	0.95	0.440
RH. Temperature	4	4.44	1.11	0.18	0.949
RH. Storage time	16	12.92	3.23	0.52	0.724
Temperature. Storage time	4	77.66	4.85	0.78	0.708
RH. Temperature. Storage time	16	100.85	6.30	1.01	0.455
Residual	100	625.11	6.25		
Total	299	869.82			

Table A91. h° of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	2457.3	614.3	3.34	0.013
Temperature	1	671.7	671.7	3.65	0.049
Storage time	4	870.8	217.7	1.18	0.323
RH. Temperature	4	413.6	103.4	0.56	0.691
RH. Storage time	16	1352.1	84.5	0.46	0.960
Temperature. Storage time	4	1708.5	427.1	2.32	0.062
RH. Temperature. Storage time	16	4004.3	250.3	1.36	0.177
Residual	100	18396.7	184.0		
Total	299	29874.9			

Table A92. h° of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	143.1	35.8	0.21	0.930
Temperature	1	40.5	40.5	0.24	0.624
Storage time	4	2212.8	553.2	3.31	0.104
RH. Temperature	4	115.5	28.9	0.17	0.952
RH. Storage time	16	2228.4	26.1	0.16	0.960
Temperature. Storage time	4	104.6	139.3	0.83	0.644
RH. Temperature. Storage time	16	2958.7	184.9	1.11	0.359
Residual	100	16694.2			
Total	299	24497.7			

Table A93. TSS of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	1.40	0.35	0.30	0.879
Temperature	1	1.60	1.60	1.36	0.246
Storage time	4	10.24	2.56	2.17	0.077
RH. Temperature	4	3.76	0.94	0.80	0.529
RH. Storage time	16	10.16	2.54	2.16	0.079
Temperature. Storage time	4	21.51	1.34	1.14	0.329
RH. Temperature. Storage time	16	21.31	1.33	1.13	0.338
Residual	100	117.75	1.18		
Total	299	187.73			

Table A94. TSS of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	2.60	0.65	0.85	0.495
Temperature	1	1.46	1.46	1.91	0.170
Storage time	4	2.30	0.57	0.75	0.558
RH. Temperature	4	1.08	0.27	0.36	0.840
RH. Storage time	16	13.51	0.84	1.11	0.360
Temperature. Storage time	4	3.55	0.89	1.16	0.333
RH. Temperature. Storage time	16	14.35	0.90	1.18	0.301
Residual	100	76.33	0.76		
Total	299	115.19			

Table A95. Aril sucrose concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	49587.15	6932.18	2712.00	<.001
Temperature	1	27729.18	49587.18	19399.16	<.001
Storage time	4	29454.83	7363.71	2880.78	<.001
RH. Temperature	4	5451.02	1362.76	533.13	<.001
RH. Storage time	16	1151.70	287.93	112.64	<.001
Temperature. Storage time	4	1041.02	65.06	25.45	<.001
RH. Temperature. Storage time	16	3039.63	189.98	74.32	<.001
Residual	100	255.61	2.56		
Total	149	117710.16			

Table A96. Aril sucrose concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	10476.37	2616.09	31.23	<.001
Temperature	1	149876.92	149876.92	1787.25	<.001
Storage time	4	89933.63	22483.41	268.11	<.001
RH. Temperature	4	2873.65	718.57	8.57	<.001
RH. Storage time	16	11865.10	741.57	8.84	<.001
Temperature. Storage time	4	7506.08	1876.72	22.38	<.001
RH. Temperature. Storage time	16	6495.26	405.95	4.84	<.001
Residual	100	8385.90	83.86		
Total	149	287413.70			

Table A97. Aril glucose concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	50888.70	73915.79	1216.95	<.001
Temperature	1	73915.79	12722.17	7070.46	<.001
Storage time	4	8416.89	2104.22	201.28	<.001
RH. Temperature	4	49394.68	12348.67	1181.22	<.001
RH. Storage time	16	1220.22	305.05	29.18	<.001
Temperature. Storage time	4	2318.00	144.87	13.86	<.001
RH. Temperature. Storage time	16	1531.90	95.74	9.16	<.001
Residual	100	1045.42	10.45		
Total	149	188731.59			

Table A98. Aril glucose concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	3089.68	772.42	13.54	<.001
Temperature	1	1227.97	1227.97	21.53	<.001
Storage time	4	14114.47	3528.62	61.86	<.001
RH. Temperature	4	1534.91	383.73	6.73	<.001
RH. Storage time	16	6676.92	417.31	7.32	<.001
Temperature. Storage time	4	3163.47	790.87	13.86	<.001
RH. Temperature. Storage time	16	5427.74	339.23	5.95	<.001
Residual	100	5704.58	57.05		
Total	149	40939.74			

Table A99. Aril fructose concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	22988.77	5747.19	629.51	<.001
Temperature	1	53977.70	53977.70	5912.37	<.001
Storage time	4	9951.49	2487.87	272.51	<.001
RH. Temperature	4	23325.14	5831.29	638.72	<.001
RH. Storage time	16	671.31	167.83	18.38	<.001
Temperature. Storage time	4	1403.15	87.70	9.61	<.001
RH. Temperature. Storage time	16	1537.01	96.06	10.52	<.001
Residual	100	912.96	9.13		
Total	149	114767.53			

Table A100. Aril fructose concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	4932.78	1233.19	19.52	<.001
Temperature	1	894.90	894.90	14.16	<.001
Storage time	4	35320.71	8830.18	139.74	<.001
RH. Temperature	4	2128.98	532.25	8.42	<.001
RH. Storage time	16	3807.08	237.94	3.77	<.001
Temperature. Storage time	4	2905.66	726.42	11.50	<.001
RH. Temperature. Storage time	16	5343.00	333.94	5.28	<.001
Residual	100	6319.19	63.19		
Total	149	61652.29			

Table A101. Pericarp glucose concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	1049.72	262.43	19.72	<.001
Temperature	1	310.34	310.34	23.32	<.001
Storage time	4	6635.59	1658.90	124.64	<.001
RH. Temperature	4	752.06	188.02	14.13	<.001
RH. Storage time	16	617.28	38.58	2.90	<.001
Temperature. Storage time	4	620.82	155.21	11.66	<.001
RH. Temperature. Storage time	16	1602.58	100.16	7.53	<.001
Residual	100	1330.92	13.31		
Total	149	12919.31			

Table A102. Pericarp glucose concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	127.72	31.93	14.73	<.001
Temperature	1	306.85	306.85	141.53	<.001
Storage time	4	3124.26	781.07	360.26	<.001
RH. Temperature	4	425.94	106.49	49.12	<.001
RH. Storage time	16	3722.24	232.64	107.30	<.001
Temperature. Storage time	4	1851.96	462.99	213.55	<.001
RH. Temperature. Storage time	16	2548.42	159.28	73.46	<.001
Residual	100	216.81	2.168		
Total	149	12324.20			

Table A103. Pericarp mannose concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	116.75	29.19	1.06	0.038
Temperature	1	525.20	525.20	19.00	<.001
Storage time	4	170.04	42.51	1.54	0.019
RH. Temperature	4	101.16	25.29	0.91	0.045
RH. Storage time	16	472.68	29.54	1.07	0.039
Temperature. Storage time	4	62.60	15.65	0.57	0.068
RH. Temperature. Storage time	16	404.45	25.28	0.91	0.050
Residual	100	2764.89	27.65		
Total	149	4617.77			

Table A104. Pericarp mannose concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	1429.54	357.38	54.62	<.001
Temperature	1	166.97	166.97	25.52	<.001
Storage time	4	50.45	12.61	1.93	0.012
RH. Temperature	4	699.29	174.82	26.72	<.001
RH. Storage time	16	275.18	17.20	2.63	0.002
Temperature. Storage time	4	305.70	76.43	11.68	<.001
RH. Temperature. Storage time	16	266.98	16.67	2.55	0.002
Residual	100	654.31	6.54		
Total	149	3848.44			

Table A105. Pericarp fructose concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	606.40	151.60	11.97	<.001
Temperature	1	23.37	23.37	1.84	0.017
Storage time	4	5820.02	1455.01	114.87	<.001
RH. Temperature	4	591.23	147.81	11.67	<.001
RH. Storage time	16	1457.73	91.11	7.19	<.001
Temperature. Storage time	4	431.40	107.85	8.51	<.001
RH. Temperature. Storage time	16	2955.11	184.69	14.58	<.001
Residual	100	1266.62	12.67		
Total	149	13151.89			

Table A106. Pericarp fructose concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	475.261	118.82	46.20	<.001
Temperature	1	882.53	882.53	343.14	<.001
Storage time	4	4338.76	1084.69	421.74	<.001
RH. Temperature	4	592.86	148.22	57.63	<.001
RH. Storage time	16	3917.97	244.87	95.21	<.001
Temperature. Storage time	4	2263.98	565.99	220.07	<.001
RH. Temperature. Storage time	16	2622.52	163.91	63.73	<.001
Residual	100	257.19	2.57		
Total	149	15351.09			

Table A107. Ascorbic acid concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	2.54	0.64	265.02	0.040
Temperature	1	0.01	0.01	4.31	<.001
Storage time	4	5.41	1.35	564.21	<.001
RH. Temperature	4	5.27	1.31	549.35	<.001
RH. Storage time	16	7.34	0.12	191.24	<.001
Temperature. Storage time	4	0.50	0.27	52.07	<.001
RH. Temperature. Storage time	16	4.36	0.45	113.77	<.001
Residual	100	0.24	0.002		
Total	149	25.69			

Table A108. Ascorbic acid concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	2.57	0.64	56.10	<.001
Temperature	1	3.69	3.69	321.75	<.001
Storage time	4	1.02	0.26	22.33	<.001
RH. Temperature	4	2.87	0.71	62.45	<.001
RH. Storage time	16	2.60	0.16	14.18	<.001
Temperature. Storage time	4	2.46	0.61	53.55	<.001
RH. Temperature. Storage time	16	5.57	0.34	30.34	<.001
Residual	100	1.15	0.01		
Total	149	21.93			

Table A109. Citric concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	510.68	127.67	243.51	<.001
Temperature	1	271.35	271.35	516.51	<.001
Storage time	4	35.73	8.93	17.00	<.001
RH. Temperature	4	728.30	182.07	346.58	<.001
RH. Storage time	16	30.71	7.67	14.62	<.001
Temperature. Storage time	4	61.85	3.86	7.36	<.001
RH. Temperature. Storage time	16	65.26	4.07	7.76	<.001
Residual	100	52.53	0.52		
Total	149	1756.44			

Table A110. Citric concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	64.91	16.23	79.94	<.001
Temperature	1	14.65	14.65	72.18	<.001
Storage time	4	6.43	1.61	7.92	<.001
RH. Temperature	4	51.46	12.87	63.38	<.001
RH. Storage time	16	5.63	0.35	1.74	0.050
Temperature. Storage time	4	2.16	0.54	2.67	0.037
RH. Temperature. Storage time	16	10.50	0.66	3.23	<.001
Residual	100	20.30	0.20		
Total	149	176.06			

Table A111. Malic acid concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	214.40	53.60	43.23	<.001
Temperature	1	127.21	127.21	102.59	<.001
Storage time	4	64.74	16.19	13.06	<.001
RH. Temperature	4	191.75	47.93	38.65	<.001
RH. Storage time	16	357.06	89.27	71.99	<.001
Temperature. Storage time	4	158.45	39.48	31.84	<.001
RH. Temperature. Storage time	16	283.01	17.69	14.27	<.001
Residual	100	123.49	1.24		
Total	149	632.04			

Table A112. Malic acid concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	49.07	12.27	19.75	<.001
Temperature	1	53.02	53.02	85.35	<.001
Storage time	4	50.05	12.51	20.14	<.001
RH. Temperature	4	78.45	19.61	31.57	<.001
RH. Storage time	16	77.91	4.87	7.84	<.001
Temperature. Storage time	4	18.54	4.64	7.46	<.001
RH. Temperature. Storage time	16	75.68	4.73	7.61	<.001
Residual	100	62.12	0.62		
Total	149	464.85			

Table A113. Oxalic acid concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	17.63	4.41	143.78	<.001
Temperature	1	0.85	0.85	742.67	<.001
Storage time	4	4.14	1.04	174.51	<.001
RH. Temperature	4	34.18	8.54	1439.47	<.001
RH. Storage time	16	2.59	0.64	109.27	<.001
Temperature. Storage time	4	13.49	0.84	142.00	<.001
RH. Temperature. Storage time	16	6.14	0.38	64.61	<.001
Residual	100	0.59	0.006		
Total	149	79.62			

Table A114. Oxalic acid concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	0.16	0.04	45.84	<.001
Temperature	1	0.04	0.04	47.95	<.001
Storage time	4	0.59	0.15	172.01	<.001
RH. Temperature	4	0.20	0.05	58.02	<.001
RH. Storage time	16	1.04	0.07	75.97	<.001
Temperature. Storage time	4	0.16	0.04	45.81	<.001
RH. Temperature. Storage time	16	2.29	0.14	166.68	<.001
Residual	100	0.09	0.0008		
Total	149	4.56			

Table A115. Tartaric acid concentration of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	21.63	4.40	742.67	<.001
Temperature	1	0.85	0.85	143.78	<.001
Storage time	4	4.14	1.035	174.51	<.001
RH. Temperature	4	34.18	8.54	1439.47	<.001
RH. Storage time	16	2.59	0.64	109.27	<.001
Temperature. Storage time	4	13.48	0.84	142.00	<.001
RH. Temperature. Storage time	16	6.13	0.38	64.61	<.001
Residual	100	0.59	0.0005		
Total	149	89.65			

Table A116. Tartaric acid concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	33.15	8.29	7.53	<.001
Temperature	1	1.08	1.08	0.98	0.032
Storage time	4	276.03	69.01	62.73	<.001
RH. Temperature	4	36.34	9.09	8.26	<.001
RH. Storage time	16	229.08	14.32	13.01	<.001
Temperature. Storage time	4	91.79	22.95	20.86	<.001
RH. Temperature. Storage time	16	310.82	19.43	17.66	<.001
Residual	100	110.02	1.10		
Total	149	1088.31			

Table A117. Ascorbic acid concentration in pericarp of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	47.02	12.27	19.75	0.007
Temperature	1	53.02	53.02	85.35	<.001
Storage time	4	50.05	12.51	20.14	<.001
RH. Temperature	4	78.45	19.61	31.57	<.001
RH. Storage time	16	77.91	4.87	7.84	<.001
Temperature. Storage time	4	18.54	4.64	7.46	<.001
RH. Temperature. Storage time	16	75.68	4.73	7.61	<.001
Residual	100	62.12	0.62		
Total	149	464.85			

Table A118. Ascorbic acid concentration in pericarp of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	16.53	4.40	712.67	<.001
Temperature	1	0.85	0.85	143.78	<.001
Storage time	4	4.14	1.11	174.51	<.001
RH. Temperature	4	34.18	8.54	1439.47	<.001
RH. Storage time	16	2.59	0.64	109.27	<.001
Temperature. Storage time	4	13.48	0.84	142.00	<.001
RH. Temperature. Storage time	16	6.13	0.38	64.61	<.001
Residual	100	0.59	0.15		
Total	149	70.42			

Table A119. Citric acid concentration in pericarp of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	64.91	16.23	79.94	<.001
Temperature	1	14.65	14.65	72.18	<.001
Storage time	4	6.43	1.61	7.92	<.001
RH. Temperature	4	51.46	12.87	63.38	0.005
RH. Storage time	16	5.63	0.35	1.74	<.001
Temperature. Storage time	4	2.16	0.54	2.67	<.001
RH. Temperature. Storage time	16	10.50	0.66	3.23	<.001
Residual	100	20.30	0.20		
Total	149	176.06			

Table A120. Citric acid concentration in pericarp of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	21.63	4.40	742.67	0.002
Temperature	1	0.85	0.85	143.78	<.001
Storage time	4	4.14	1.035	174.51	<.001
RH. Temperature	4	34.18	8.54	1439.47	<.001
RH. Storage time	16	2.59	0.64	109.27	<.001
Temperature. Storage time	4	13.48	0.84	142.00	<.001
RH. Temperature. Storage time	16	6.13	0.38	64.61	<.001
Residual	100	0.59	0.0005		
Total	149	89.65			

Table A121. Malic acid concentration in pericarp of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	34.91	16.23	79.94	<.001
Temperature	1	14.65	14.65	72.18	<.001
Storage time	4	6.43	1.61	7.92	<.001
RH. Temperature	4	28.99	7.25	2.99	<.001
RH. Storage time	16	18.87	5.65	2.38	0.045
Temperature. Storage time	4	33.74	8.44	3.49	<.001
RH. Temperature. Storage time	16	20.64	6.88	6.44	<.001
Residual	100	36.26	2.42		
Total	149	51.26			

Table A122. Malic acid concentration in pericarp of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	49.07	12.27	19.75	<.001
Temperature	1	53.02	53.02	85.35	<.001
Storage time	4	50.05	12.51	20.14	<.001
RH. Temperature	4	78.45	19.61	31.57	<.001
RH. Storage time	16	77.91	4.87	7.84	<.001
Temperature. Storage time	4	18.54	4.64	7.46	<.001
RH. Temperature. Storage time	16	75.68	4.73	7.61	<.001
Residual	100	62.12	0.62		
Total	149	324.85			

Table A123. Oxalic acid concentration in pericarp of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	22.68	5.67	94.50	<.001
Temperature	1	0.85	0.85	742.67	<.001
Storage time	4	4.14	1.04	174.51	<.001
RH. Temperature	4	34.18	8.54	399.34	<.001
RH. Storage time	16	2.59	0.64	109.27	<.001
Temperature. Storage time	4	13.49	0.84	142.00	<.001
RH. Temperature. Storage time	16	6.14	0.38	64.61	<.001
Residual	100	0.59	0.06		
Total	149	91.55			

Table A124. Oxalic acid concentration in pericarp of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	21.50	4.40	510.02	<.001
Temperature	1	0.85	0.85	143.78	<.001
Storage time	4	4.14	1.11	174.51	<.001
RH. Temperature	4	34.18	8.54	449.46	<.001
RH. Storage time	16	2.59	0.64	109.27	<.001
Temperature. Storage time	4	13.48	0.84	142.00	<.001
RH. Temperature. Storage time	16	6.13	0.38	64.69	<.001
Residual	100	0.59	0.15		
Total	149	81.53			

Table A125. Tartaric acid concentration in pericarp of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	39.07	10.27	13.75	<.001
Temperature	1	53.02	53.02	85.35	<.001
Storage time	4	50.05	12.51	20.14	<.001
RH. Temperature	4	78.45	19.61	31.57	<.001
RH. Storage time	16	77.91	4.87	7.84	<.001
Temperature. Storage time	4	18.54	4.64	7.46	<.001
RH. Temperature. Storage time	16	75.68	4.73	8.01	<.001
Residual	100	62.12	0.62		
Total	149	244.00			

Table A126. Tartaric acid concentration of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	64.91	16.23	79.94	<.001
Temperature	1	14.65	14.65	72.18	<.001
Storage time	4	6.43	1.61	7.92	<.001
RH. Temperature	4	51.46	12.87	63.38	<.001
RH. Storage time	16	5.63	0.35	1.74	<.001
Temperature. Storage time	4	2.16	0.54	2.67	<.001
RH. Temperature. Storage time	16	10.50	0.66	3.23	<.001
Residual	100	20.30	0.20		
Total	149	176.06			

Table A127. Cyanidin 3-glucoside of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	149.58	37.40	16.91	<.001
Temperature	1	1212.79	1212.79	548.35	<.001
Storage time	4	537.84	134.46	60.79	<.001
RH. Temperature	4	113.83	28.46	12.87	<.001
RH. Storage time	16	1180.29	73.77	33.35	<.001
Temperature. Storage time	4	198.99	49.75	22.49	<.001
RH. Temperature. Storage time	16	1831.08	114.44	51.74	<.001
Residual	100	221.17	2.21		
Total	149	5445.57			

Table A128. Cyanidin 3-glucoside of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	2104.79	526.20	10.63	<.001
Temperature	1	16784.15	16784.15	339.02	<.001
Storage time	4	17504.47	4376.12	88.39	<.001
RH. Temperature	4	2909.39	727.35	14.69	<.001
RH. Storage time	16	7847.62	490.48	9.91	<.001
Temperature. Storage time	4	2529.07	632.27	12.77	<.001
RH. Temperature. Storage time	16	11875.59	742.22	14.99	<.001
Residual	100	4950.84	49.51		
Total	149	66505.92			

Table A129. Cyanidin 3-rutinoside of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	1370476	19775644	941.60	<.001
Temperature	1	19775644	342619	16.31	<.001
Storage time	4	5758598	1439649	68.55	<.001
RH. Temperature	4	1453104	363276	17.30	<.001
RH. Storage time	16	8101607	693777	33.03	<.001
Temperature. Storage time	4	2775108	506350	24.11	<.001
RH. Temperature. Storage time	16	11385214	711576	33.88	<.001
Residual	100	2100208	21002		
Total	149	52719958			

Table A130. Cyanidin 3-rutinoside of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	218207	54552	35.65	<.001
Temperature	1	243800	243800	159.31	<.001
Storage time	4	3024872	756218	494.15	<.001
RH. Temperature	4	157207	39302	25.68	<.001
RH. Storage time	16	1064885	66555	43.49	<.001
Temperature. Storage time	4	997143	249286	162.90	<.001
RH. Temperature. Storage time	16	1534903	95931	62.69	<.001
Residual	100	153034	1530		
Total	149	7394052			

Table A131. Malvidin 3-glucoside of Experiment 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	126.74	31.68	26.53	<.001
Temperature	1	901.06	901.06	754.66	<.001
Storage time	4	396.26	99.07	82.95	<.001
RH. Temperature	4	113.33	28.33	23.72	<.001
RH. Storage time	16	810.70	50.67	42.43	<.001
Temperature. Storage time	4	243.80	60.95	51.04	<.001
RH. Temperature. Storage time	16	743.14	46.45	38.89	<.001
Residual	100	119.43	1.194		
Total	149	3454.46			

Table A132. Malvidin-3-glucoside of Experiment 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
RH	4	105.21	26.30	65.46	<.001
Temperature	1	98.65	98.66	245.56	<.001
Storage time	4	272.10	68.03	169.31	<.001
RH. Temperature	4	45.13	11.28	28.08	<.001
RH. Storage time	16	148.45	9.28	23.09	<.001
Temperature. Storage time	4	475.26	118.82	295.72	<.001
RH. Temperature. Storage time	16	485.51	30.34	75.52	<.001
Residual	100	40.18	0.40		
Total	149	1670.51			

Table A133. CO_2 concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	3945.97	986.49	508.73	<.001
Storage time	4	844.29	211.07	108.85	<.001
Packaging materials. Storage time	16	1218.23	76.14	39.26	<.001
Residual	50	96.96	1.94		
Total	74	6105.44			

Table A134. Ethylene concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	53.34	13.34	85.98	<.001
Storage time	4	14.20	3.55	22.89	<.001
Packaging materials. Storage time	16	25.18	1.57	10.15	<.001
Residual	50	7.75	0.16		
Total	74	100.48			

Table A135. Weight loss of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	1238.50	309.62	687.17	<.001
Storage time	4	981.59	245.40	544.63	<.001
Packaging materials. Storage time	16	462.45	28.90	64.15	<.001
Residual	425	191.49	0.45		
Total	449	2874.03			

Table A136. Aril dry matter of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	20.34	5.08	3.06	0.017
Storage time	4	24.11	6.03	3.63	0.006
Packaging materials. Storage time	16	46.59	2.91	1.75	0.035
Residual	425	705.28	1.66		
Total	449	796.31			

Table A137. Aril moisture content of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	20.34	5.08	3.06	0.017
Storage time	4	24.11	6.03	3.63	0.006
Packaging materials. Storage time	16	46.59	2.91	1.75	0.035
Residual	425	705.28	1.66		
Total	449	796.31			

Table A138. Pericarp dry matter of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	39578.07	9894.52	319.09	<.001
Storage time	4	6233.29	1558.32	50.25	<.001
Packaging materials. Storage time	16	10740.58	671.29	21.65	<.001
Residual	425	13178.73	31.01		
Total	449	69730.67			

Table A139. Pericarp moisture content of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	39578.07	9894.52	319.09	<.001
Storage time	4	6233.29	1558.32	50.25	<.001
Packaging materials. Storage time	16	10740.58	671.29	21.65	<.001
Residual	425	13178.73	31.01		
Total	449	69730.67			

Table A140. Total soluble solids of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	15.15	3.79	2.97	0.019
Storage time	4	27.62	6.91	5.42	<.001
Packaging materials. Storage time	16	14.62	0.91	0.72	0.017
Residual	425	541.48	1.27		
Total	449	598.87			

Table A141. Lightness (L*) in pericarp of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	88.75	222.19	9.92	<.001
Storage time	4	3425.35	855.84	38.23	<.001
Packaging materials. Storage time	16	383.24	23.95	1.07	0.038
Residual	425	9515.05	22.39		
Total	449	14210.39			

Table A142. Colour intensity (C*) in pericarp of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	250.39	62.60	13.21	<.001
Storage time	4	564.57	141.14	29.78	<.001
Packaging materials. Storage time	16	107.27	6.70	1.41	0.013
Residual	425	2014.07	4.74		
Total	449	2936.30			

Table A143. Hue angle (h°) in pericarp of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	463.10	115.77	1.20	0.031
Storage time	4	2545.52	636.38	6.58	<.001
Packaging materials. Storage time	16	932.97	58.31	0.60	0.028
Residual	425	41096.23	96.70		
Total	449	45037.81			

Table A144. Aril sucrose concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	4654.80	1163.70	3.09	0.026
Storage time	4	14823.30	4941.10	13.11	<.001
Packaging materials. Storage time	16	3945.30	328.80	0.87	0.050
Residual	425	15075.40	376.90		
Total	449	38498.70			

Table A145. Aril glucose concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	382.20	95.60	0.36	0.032
Storage time	4	1463.90	488.00	1.86	0.035
Packaging materials. Storage time	16	1774.40	147.90	0.56	0.047
Residual	200	10473.60	261.80		
Total	224	14094.10			

Table A146. Aril fructose concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	611.10	152.80	0.47	0.042
Storage time	4	3985.00	1328.30	4.06	<.001
Packaging materials. Storage time	16	1884.20	157.00	0.48	0.015
Residual	200	13095.00	327.40		
Total	224	19575.30			

Table A147. Aril total sugar concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	29069.27	7267.29	8.33	<.001
Storage time	4	51815.30	17271.82	19.81	<.001
Packaging materials. Storage time	16	47170.71	3930.89	4.51	<.001
Residual	200	34877.66	871.90		
Total	224	162933.00			

Table A148. Pericarp glucose concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	2156.95	539.24	16.70	<.001
Storage time	4	3068.28	1022.76	31.67	<.001
Packaging materials. Storage time	16	3110.78	259.23	8.03	<.001
Residual	200	5167.64	32.30		
Total	224	13503.65			

Table A149. Pericarp mannose concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	354.51	88.63	3.92	0.005
Storage time	4	698.17	232.72	10.28	<.001
Packaging materials. Storage time	16	1764.49	147.04	6.50	<.001
Residual	200	3621.12	22.63		
Total	224	6438.29			

Table A150. Pericarp fructose concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	584.03	146.01	22.43	<.001
Storage time	4	218.10	72.70	11.17	<.001
Packaging materials. Storage time	16	659.15	54.93	8.44	<.001
Residual	200	1041.48	6.51		
Total	224	2502.77			

Table A151. Pericarp total sugar concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	3129.10	782.27	9.04	<.001
Storage time	4	549.71	183.24	2.12	0.100
Packaging materials. Storage time	16	10514.07	876.17	10.13	<.001
Residual	200	13841.90	86.51		
Total	224	28034.78			

Table A152. Aril tartaric acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	34.46	8.62	5.12	0.002
Storage time	4	10.46	2.62	1.55	0.201
Packaging materials. Storage time	16	18.15	1.13	0.67	0.085
Residual	200	84.18	1.68		
Total	224	147.257			

Table A153. Aril oxalic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	0.039	0.010	0.67	0.617
Storage time	4	0.097	0.024	1.66	0.175
Packaging materials × Storage time	16	0.172	0.011	0.74	0.045
Residual	200	0.731	0.015		
Total	224	1.039			

Table A154. Aril malic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	40.48	10.12	5.50	<.001
Storage time	4	119.29	29.82	16.22	<.001
Packaging materials × Storage time	16	35.15	2.197	1.19	0.035
Residual	200	91.91	1.84		
Total	224	286.82			

Table A155. Aril ascorbic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	0.47	0.12	2.33	0.049
Storage time	4	2.27	0.57	11.32	<.001
Packaging materials × Storage time	16	1.42	0.09	1.78	0.050
Residual	200	2.50	0.05		
Total	224	6.67			

Table A156. Aril citric acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	20.09	5.02	5.97	<.001
Storage time	4	35.59	8.90	10.58	<.001
Packaging materials × Storage time	16	28.00	1.75	2.08	0.025
Residual	200	42.07	0.84		
Total	224	125.76			

Table A157. Aril total acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	64.46	16.12	5.48	<.001
Storage time	4	389.63	97.41	33.13	<.001
Packaging materials × Storage time	16	86.56	5.41	1.84	0.050
Residual	200	147.01	2.94		
Total	224	687.66			

Table A158. Pericarp tartaric acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	55.09	13.77	1.43	0.224
Storage time	4	109.03	27.26	2.84	<.001
Packaging materials × Storage time	16	341.33	21.33	2.22	0.006
Residual	200	1920.63	9.60		
Total	224	2426.08			

Table A159. Pericarp oxalic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	30.21	7.55	7.10	<.001
Storage time	4	228.95	57.24	53.81	<.001
Packaging materials × Storage time	16	76.95	4.81	4.52	<.001
Residual	200	212.75	1.06		
Total	224	548.85			

Table A160. Pericarp malic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	28.36	7.09	1.58	0.018
Storage time	4	288.70	72.17	16.04	<.001
Packaging materials × Storage time	16	248.02	15.50	3.45	<.001
Residual	200	899.77	4.50		
Total	224	1464.86			

Table A161. Pericarp ascorbic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	0.09	0.22	1.63	0.168
Storage time	4	3.15	0.79	56.48	<.001
Packaging materials × Storage time	16	0.40	0.02	1.79	0.035
Residual	200	2.79	0.01		
Total	224	6.44			

Table A162. Pericarp citric acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	373.74	93.43	19.99	<.001
Storage time	4	423.26	105.81	22.64	<.001
Packaging materials × Storage time	16	537.33	33.58	7.19	<.001
Residual	200	934.72	4.67		
Total	224	2269.05			

Table A163. Pericarp total organic acid concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	421.56	105.39	3.53	0.008
Storage time	4	3160.11	790.03	26.46	<.001
Packaging materials × Storage time	16	2729.68	170.60	5.71	<.001
Residual	200	5971.72	29.86		
Total	224	12283.08			

Table A164. Cyanidin 3-glucoside concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	8851.50	2212.90	8.55	<.001
Storage time	4	6548.50	1637.10	6.33	<.001
Packaging materials × Storage time	16	9253.10	578.30	2.24	0.005
Residual	200	51747.40	258.70		
Total	224	76400.40			

Table A165. Cyanidin 3-rutinoside concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	419359	104840	4.60	0.001
Storage time	4	581949	145487	6.38	<.001
Packaging materials × Storage time	16	646239	40390	1.77	0.037
Residual	200	4561209	22806		
Total	224	6208757			

Table A166. Malvidin 3-glucoside concentration of litchi fruit of Experiment 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Packaging materails	4	20.50	5.12	1.25	0.029
Storage time	4	169.23	42.31	10.32	<.001
Packaging materials × Storage time	16	124.45	7.78	1.90	0.022
Residual	200	819.70	4.10		
Total	224	1133.88			

Table A167. Weight loss of litchi fruit of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	117.10	117.10	52.48	<.001
Packaging materials	2	4170.10	2085.05	934.44	<.001
Chemical treatment	1	57.41	57.41	25.73	<.001
Storage time	2	672.47	336.23	150.69	<.001
Temperature.Packaging materials	2	22.65	11.32	5.07	0.007
Temperature.Chemical treatment.	1	21.68	21.69	9.72	0.002
Packaging materials.Chemical treatment	2	107.15	53.57	24.01	<.001
Temperature. Storage time	2	41.66	20.83	9.34	<.001
Packaging materials. Storage time	4	416.45	104.11	46.66	<.001
Chemical treatment. Storage time	2	4.18	2.09	0.94	0.392
Temperature. Packaging materials. Chemical treatment	2	16.41	8.21	3.68	0.026
Temperature. Packaging materials. Storage time	4	21.86	5.47	2.45	0.045
Temperature. Chemical treatment. Storage time	2	7.34	3.67	1.65	0.194
Packaging materials. Chemical treatment. Storage time	4	13.20	3.30	1.48	0.207
Temperature. Packaging materials. Chemical treatment. Storage time	4	8.58	2.14	0.96	0.043
Residual	612	1365.58	2.23		
Total	647	7063.83			

Table A164. Pericarp moisture content of litchi fruit of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	18339.64	18339.64	380.10	<.001
Packaging materials	2	75829.39	37914.69	785.80	<.001
Chemical treatment	1	1379.73	1379.73	28.60	<.001
Storage time	2	328.79	164.39	3.41	0.034
Temperature.Packaging materials	2	6678.89	3339.44	69.21	<.001
Temperature.Chemical treatment.	1	56.79	56.79	1.18	0.278
Packaging materials.Chemical treatment	2	501.55	250.78	5.20	0.006
Temperature. Storage time	2	1047.02	523.51	10.85	<.001
Packaging materials. Storage time	4	1725.91	431.48	8.94	<.001
Chemical treatment. Storage time	2	560.61	280.30	5.81	0.003
Temperature. Packaging materials. Chemical treatment	2	195.88	97.94	2.03	0.132
Temperature. Packaging materials. Storage time	4	192.50	48.12	1.00	0.408
Temperature. Chemical treatment. Storage time	2	314.82	157.41	3.26	0.039
Packaging materials. Chemical treatment. Storage time	4	1002.94	250.73	5.20	<.001
Temperature. Packaging materials. Chemical treatment. Storage time	4	868.78	217.20	4.50	0.001
Residual	612	29529.06	48.25		
Total	647	138552.29			

Table A165. Lightness (L*) of litchi fruit of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	2.71	2.71	0.15	0.072
Packaging materials	2	3225.36	1612.68	87.17	<.001
Chemical treatment	1	8974.58	8974.58	485.11	<.001
Storage time	2	255.25	127.62	6.90	0.001
Temperature.Packaging materials	2	14.48	7.24	0.39	0.676
Temperature.Chemical treatment.	1	1.44	1.44	0.08	0.780
Packaging materials.Chemical treatment	2	70.79	35.40	1.91	0.148
Temperature. Storage time	2	38.62	19.31	1.04	0.353
Packaging materials. Storage time	4	81.95	20.49	1.11	0.352
Chemical treatment. Storage time	2	11.17	5.58	0.30	0.740
Temperature. Packaging materials. Chemical treatment	2	20.60	10.30	0.56	0.573
Temperature. Packaging materials. Storage time	4	288.01	72.00	3.89	0.004
Temperature. Chemical treatment. Storage time	2	18.50	9.27	0.50	0.606
Packaging materials. Chemical treatment. Storage time	4	83.78	20.94	1.13	0.340
Temperature. Packaging materials. Chemical treatment. Storage time	4	7.65	1.91	0.10	0.981
Residual	612	11322.05	18.50		
Total	647	24416.98			

Table A166. Colour intensity (C*) of litchi fruit of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	900.29	450.14	37.59	<.001
Packaging materials	2	13922.80	13922.80	1162.78	<.001
Chemical treatment	1	673.28	673.28	56.23	<.001
Storage time	2	296.26	148.13	12.37	<.001
Temperature.Packaging materials	2	1374.51	687.26	57.40	<.001
Temperature.Chemical treatment.	1	8.02	4.01	0.33	0.716
Packaging materials.Chemical treatment	2	74.43	74.43	6.22	0.013
Temperature. Storage time	2	82.78	20.69	1.73	0.142
Packaging materials. Storage time	4	326.55	163.27	13.64	<.001
Chemical treatment. Storage time	2	76.46	38.23	3.19	0.042
Temperature. Packaging materials. Chemical treatment	2	14.65	7.33	0.61	0.543
Temperature. Packaging materials. Storage time	4	55.35	13.84	1.16	0.329
Temperature. Chemical treatment. Storage time	2	62.57	15.64	1.31	0.266
Packaging materials. Chemical treatment. Storage time	4	7.75	3.87	0.32	0.724
Temperature. Packaging materials. Chemical treatment. Storage time	4	106.85	26.71	2.23	0.064
Residual	612	7327.92	11.97		
Total	647	25310.47			

Table A167. Hue angle (h°) of litchi fruit of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	438.69	438.69	10.20	0.001
Packaging materials	2	620.20	310.10	7.21	<.001
Chemical treatment	1	241.99	241.99	56.13	<.001
Storage time	2	35.49	17.75	0.41	0.062
Temperature.Packaging materials	2	12.50	6.25	0.15	0.165
Temperature.Chemical treatment.	1	169.99	169.99	3.95	0.047
Packaging materials.Chemical treatment	2	3495.74	1747.87	40.62	<.001
Temperature. Storage time	2	49.41	24.70	0.57	0.363
Packaging materials. Storage time	4	273.05	68.26	1.59	0.176
Chemical treatment. Storage time	2	125.11	62.55	1.45	0.234
Temperature. Packaging materials. Chemical treatment	2	295.66	147.83	3.44	0.033
Temperature. Packaging materials. Storage time	4	193.10	48.28	1.12	0.345
Temperature. Chemical treatment. Storage time	2	3.97	1.99	0.05	0.155
Packaging materials. Chemical treatment. Storage time	4	69.19	17.30	0.40	0.807
Temperature. Packaging materials. Chemical treatment. Storage time	4	106.85	26.71	2.23	0.016
Residual	612	7327.92	11.97		
Total	647	25310.47			

Table A168. CO_2 concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Chemical treatment	1	86.84	86.84	67.93	<.001
Packaging materials	2	534.88	267.44	48.19	<.001
Temperature	1	107.88	107.88	84.38	<.001
Storage time	2	44.69	22.34	17.48	<.001
Packaging materials.Chemical treatment	2	170.87	85.44	66.83	<.001
Temperature.Chemical treatment.	1	30.20	30.20	23.63	<.001
Temperature.Packaging materials	2	218.78	109.39	85.57	<.001
Chemical treatment. Storage time	2	7.92	3.96	3.10	0.050
Packaging materials. Storage time	4	88.23	22.06	17.25	<.001
Temperature. Storage time	2	43.45	21.73	16.99	<.001
Temperature. Packaging materials. Chemical treatment	2	61.33	30.67	23.99	<.001
Packaging materials. Chemical treatment. Storage time	4	14.89	3.72	2.91	0.027
Temperature. Chemical treatment. Storage time	2	10.31	5.16	4.03	0.022
Temperature. Packaging materials. Storage time	4	89.40	22.35	17.48	<.001
Temperature. Packaging materials. Chemical treatment. Storage time	4	22.21	5.55	4.34	0.003
Residual	72	92.05	1.28		
Total	107	1623.91			

Table A169. Ethylene concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Chemical treatment	1	0.22	0.22	0.10	0.029
Packaging materials	2	4.82	2.41	48.33	<.001
Temperature	1	4.57	4.57	91.69	<.001
Storage time	2	4.85	2.43	48.67	<.001
Packaging materials.Chemical treatment	2	0.05	0.02	0.49	0.614
Temperature.Chemical treatment.	1	0.20	0.20	4.00	0.049
Temperature.Packaging materials	2	2.71	1.35	27.16	<.001
Chemical treatment. Storage time	2	0.70	0.35	7.03	0.002
Packaging materials. Storage time	4	5.78	1.44	28.98	<.001
Temperature. Storage time	2	1.80	0.90	18.02	<.001
Temperature. Packaging materials. Chemical treatment	2	0.09	0.04	0.88	0.420
Packaging materials. Chemical treatment. Storage time	4	3.11	0.78	15.59	<.001
Temperature. Chemical treatment. Storage time	2	0.82	0.41	8.19	<.001
Temperature. Packaging materials. Storage time	4	4.16	1.04	20.85	<.001
Temperature. Packaging materials. Chemical treatment. Storage time	4	0.86	0.21	4.29	0.004
Residual	72	3.59	0.05		
Total	107	38.09			

Table A170. Total soluble solids (TSS) of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Chemical treatment	1	299.99	299.99	154.45	<.001
Packaging materials	2	39.38	19.69	10.14	<.001
Temperature	1	6.74	6.74	3.47	0.063
Storage time	2	0.96	0.48	0.25	0.781
Packaging materials.Chemical treatment	2	4.64	2.32	1.19	0.303
Temperature.Chemical treatment.	1	20.95	20.95	10.78	0.001
Temperature.Packaging materials	2	2.65	1.33	0.68	0.506
Chemical treatment. Storage time	2	30.16	15.08	7.76	<.001
Packaging materials. Storage time	4	11.48	2.87	1.48	0.207
Temperature. Storage time	2	2.43	1.22	0.63	0.535
Temperature. Packaging materials. Chemical treatment	2	9.56	4.78	2.46	0.086
Packaging materials. Chemical treatment. Storage time	4	20.51	5.13	2.64	0.033
Temperature. Chemical treatment. Storage time	2	7.07	3.53	1.82	0.163
Temperature. Packaging materials. Storage time	4	5.40	1.35	0.69	0.596
Temperature. Packaging materials. Chemical treatment. Storage time	4	9.80	2.45	1.26	0.284
Residual	612	1188.72	1.94		
Total	647	1660.44			

Table A171. Aril sucrose concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	13796.8	13796.8	40.89	<.001
Chemical treatment	1	25746.6	25746.6	65.35	<.001
Packaging materials	2	840.4	420.2	1.25	0.294
Storage time	2	25003.5	12501.8	37.05	<.001
Temperature.Chemical treatment.	1	320.6	320.6	0.95	0.333
Temperature.Packaging materials	2	3757.6	1878.8	5.57	0.006
Packaging materials.Chemical treatment	2	1205.6	602.8	1.79	0.025
Temperature. Storage time	2	392.1	196.0	0.58	0.562
Chemical treatment. Storage time	2	5153.2	2576.6	7.64	<.001
Packaging materials. Storage time	4	752.5	188.1	0.56	0.694
Temperature. Packaging materials. Chemical treatment	2	188.4	94.2	0.28	0.757
Temperature. Chemical treatment. Storage time	2	380.1	190.0	0.56	0.572
Temperature. Packaging materials. Storage time	4	1861.7	465.4	1.38	0.250
Packaging materials. Chemical treatment. Storage time	4	1574.0	393.5	1.17	0.333
Temperature. Packaging materials. Chemical treatment. Storage time	4	1577.8	394.4	1.17	0.054
Residual	72	24294.5	337.4		
Total	107	33856.65			

Table A172. Aril glucose concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	1338.0	1338.0	5.90	0.018
Chemical treatment	1	11679.9	11679.9	51.50	<.001
Packaging materials	2	46.1	23.1	0.10	0.024
Storage time	2	2004.1	1002.1	4.42	0.015
Temperature.Chemical treatment.	1	107.2	107.2	0.47	0.494
Temperature.Packaging materials	2	537.9	268.9	1.19	0.311
Packaging materials.Chemical treatment	2	18.2	9.1	0.04	0.010
Temperature. Storage time	2	237.6	118.8	0.52	0.595
Chemical treatment. Storage time	2	2415.9	1207.9	5.33	0.007
Packaging materials. Storage time	4	265.4	66.3	0.29	0.882
Temperature. Packaging materials. Chemical treatment	2	23.7	11.8	0.05	0.949
Temperature. Chemical treatment. Storage time	2	346.8	173.4	0.76	0.469
Temperature. Packaging materials. Storage time	4	706.6	176.7	0.78	0.543
Packaging materials. Chemical treatment. Storage time	4	476.6	119.1	0.53	0.717
Temperature. Packaging materials. Chemical treatment. Storage time	4	677.0	169.3	0.75	0.164
Residual	72	16329.4	226.8		
Total	107	37210.4			

Table A173. Aril fructose concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	5466.2	5466.2	30.37	<.001
Chemical treatment	1	14817.3	14817.3	82.32	<.001
Packaging materials	2	17.5	8.8	0.05	0.042
Storage time	2	10432.3	5216.2	20.98	<.001
Temperature.Chemical treatment.	1	409.5	409.5	2.28	0.136
Temperature.Packaging materials	2	511.8	255.9	1.42	0.248
Packaging materials.Chemical treatment	2	533.8	266.9	1.48	0.234
Temperature. Storage time	2	839.9	419.5	2.33	0.105
Chemical treatment. Storage time	2	1436.3	718.1	3.99	0.023
Packaging materials. Storage time	4	526.3	131.6	0.73	0.574
Temperature. Packaging materials. Chemical treatment	2	66.5	33.3	0.18	0.832
Temperature. Chemical treatment. Storage time	2	304.7	152.4	0.85	0.433
Temperature. Packaging materials. Storage time	4	654.0	163.5	0.91	0.464
Packaging materials. Chemical treatment. Storage time	4	633.4	158.4	0.88	0.480
Temperature. Packaging materials. Chemical treatment. Storage time	4	1258.9	314.7	1.75	0.049
Residual	72	12959.1	180.0		
Total	107	50866.7			

Table A174. Aril ascorbic acid concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	0.006	0.006	0.04	0.046
Chemical treatment	1	5.560	5.560	34.24	<.001
Packaging materials	2	1.253	0.627	3.86	0.026
Storage time	2	1.860	0.930	5.73	0.005
Temperature.Chemical treatment.	1	0.567	0.567	3.49	0.044
Temperature.Packaging materials	2	0.252	0.126	0.78	0.464
Packaging materials.Chemical treatment	2	0.077	0.039	0.24	0.789
Temperature. Storage time	2	2.066	1.033	6.36	0.003
Chemical treatment. Storage time	2	0.096	0.048	0.30	0.744
Packaging materials. Storage time	4	2.894	0.723	4.45	0.003
Temperature. Packaging materials. Chemical treatment	2	0.499	0.249	1.53	0.222
Temperature. Chemical treatment. Storage time	2	0.845	0.422	2.60	0.081
Temperature. Packaging materials. Storage time	4	0.637	0.159	0.98	0.424
Packaging materials. Chemical treatment. Storage time	4	0.153	0.038	0.24	0.917
Temperature. Packaging materials. Chemical treatment. Storage time	4	1.643	0.411	2.53	0.048
Residual	72	11.694	0.162		
Total	107	30.102			

Table A175. Aril malic acid concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	53.47	53.47	10.24	0.002
Chemical treatment	1	164.05	164.05	31.41	<.001
Packaging materials	2	13.54	6.77	1.30	0.028
Storage time	2	345.91	172.96	33.11	<.001
Temperature.Chemical treatment.	1	0.192	0.19	0.04	0.849
Temperature.Packaging materials	2	15.22	7.61	1.46	0.240
Packaging materials.Chemical treatment	2	31.98	15.99	3.06	0.043
Temperature. Storage time	2	51.12	25.56	4.89	0.010
Chemical treatment. Storage time	2	8.97	4.48	0.86	0.428
Packaging materials. Storage time	4	25.38	6.34	1.21	0.312
Temperature. Packaging materials. Chemical treatment	2	58.04	29.02	5.56	0.006
Temperature. Chemical treatment. Storage time	2	51.10	25.55	4.89	0.010
Temperature. Packaging materials. Storage time	4	49.59	12.40	2.37	0.060
Packaging materials. Chemical treatment. Storage time	4	23.13	5.78	1.11	0.360
Temperature. Packaging materials. Chemical treatment. Storage time	4	49.18	12.29	2.35	0.050
Residual	72	376.09	5.22		
Total	107	1316.94			

Table A176. Aril tartaric acid concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	14.32	14.32	12.18	<.001
Chemical treatment	1	76.23	76.23	64.82	<.001
Packaging materials	2	2.59	1.29	1.10	0.038
Storage time	2	82.97	41.49	35.28	<.001
Temperature.Chemical treatment.	1	1.29	1.29	1.10	0.298
Temperature.Packaging materials	2	18.84	9.42	8.01	<.001
Packaging materials.Chemical treatment	2	4.05	2.03	1.72	0.186
Temperature. Storage time	2	10.47	5.24	4.45	0.015
Chemical treatment. Storage time	2	6.66	3.33	2.83	0.060
Packaging materials. Storage time	4	0.96	0.24	0.20	0.935
Temperature. Packaging materials. Chemical treatment	2	8.26	44.13	3.51	0.035
Temperature. Chemical treatment. Storage time	2	4.44	2.22	1.89	0.159
Temperature. Packaging materials. Storage time	4	18.27	4.57	3.88	0.007
Packaging materials. Chemical treatment. Storage time	4	5.09	1.27	1.08	0.372
Temperature. Packaging materials. Chemical treatment. Storage time	4	23.77	5.94	5.05	0.001
Residual	72	84.67	1.18		
Total	107	362.88			

Table A177. Aril citric acid concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	6.57	6.57	4.97	0.029
Chemical treatment	1	8.66	8.66	6.55	0.013
Packaging materials	2	10.60	5.30	4.01	0.022
Storage time	2	25.58	12.79	9.68	<.001
Temperature.Chemical treatment.	1	4.47	4.47	3.38	0.070
Temperature.Packaging materials	2	1.57	0.79	0.60	0.553
Packaging materials.Chemical treatment	2	6.74	3.37	2.55	0.085
Temperature. Storage time	2	26.28	13.14	9.94	<.001
Chemical treatment. Storage time	2	15.38	7.69	5.82	0.005
Packaging materials. Storage time	4	18.30	4.58	3.46	0.012
Temperature. Packaging materials. Chemical treatment	2	13.66	6.83	5.17	0.008
Temperature. Chemical treatment. Storage time	2	27.18	13.59	10.28	<.001
Temperature. Packaging materials. Storage time	4	17.69	4.42	3.35	0.014
Packaging materials. Chemical treatment. Storage time	4	11.48	2.87	2.17	0.081
Temperature. Packaging materials. Chemical treatment. Storage time	4	18.96	4.74	3.59	0.010
Residual	72	95.17	1.32		
Total	107	308.32			

Table A178. Aril total organic acid concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	175.29	175.29	21.98	<.001
Chemical treatment	1	407.96	407.96	51.16	<.001
Packaging materials	2	16.64	8.32	1.04	0.048
Storage time	2	903.09	451.54	56.63	<.001
Temperature.Chemical treatment.	1	3.95	3.95	0.49	0.484
Temperature.Packaging materials	2	3.09	1.55	0.19	0.824
Packaging materials.Chemical treatment	2	90.18	45.09	5.65	0.005
Temperature. Storage time	2	64.72	32.36	4.06	0.021
Chemical treatment. Storage time	2	32.66	16.33	2.05	0.136
Packaging materials. Storage time	4	75.97	18.92	2.37	0.060
Temperature. Packaging materials. Chemical treatment	2	96.71	48.35	6.06	0.004
Temperature. Chemical treatment. Storage time	2	146.89	73.45	9.21	<.001
Temperature. Packaging materials. Storage time	4	94.28	23.57	2.96	0.026
Packaging materials. Chemical treatment. Storage time	4	56.20	14.05	1.76	0.146
Temperature. Packaging materials. Chemical treatment. Storage time	4	28.95	7.24	0.91	0.464
Residual	72	574.12	7.97		
Total	107	2770.39			

Table A179. Cyanidin 3-glucoside concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	12745	12745	32.70	<.001
Chemical treatment	1	7622	7622	19.56	<.001
Packaging materials	2	39358	19679	50.49	<.001
Storage time	2	76247	38124	97.82	<.001
Temperature.Chemical treatment.	1	108	108	0.28	0.599
Temperature.Packaging materials	2	999	499	1.28	0.280
Packaging materials.Chemical treatment	2	35279	17640	45.26	<.001
Temperature. Storage time	2	1502	751	1.93	0.149
Chemical treatment. Storage time	2	947	473	1.22	0.299
Packaging materials. Storage time	4	5021	1255	3.22	0.014
Temperature. Packaging materials. Chemical treatment	2	3924	1962	5.03	0.007
Temperature. Chemical treatment. Storage time	2	186	93	0.24	0.787
Temperature. Packaging materials. Storage time	4	3585	896	2.30	0.050
Packaging materials. Chemical treatment. Storage time	4	221	55	0.14	0.966
Temperature. Packaging materials. Chemical treatment. Storage time	4	3059	764	1.96	0.042
Residual	72	70153	389		
Total	107	260964			

Table A180. Cyanidin 3-rutinoside concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	1044895	1044895	30.26	<.001
Chemical treatment	1	294099	294099	8.52	0.004
Packaging materials	2	972734	486367	14.08	<.001
Storage time	2	4578366	2289183	66.29	<.001
Temperature.Chemical treatment.	1	9791	9791	0.28	0.595
Temperature.Packaging materials	2	57080	28540	0.83	0.439
Packaging materials.Chemical treatment	2	2973645	1486822	43.05	<.001
Temperature. Storage time	2	65295	32648	0.95	0.390
Chemical treatment. Storage time	2	6093	3047	0.09	0.916
Packaging materials. Storage time	4	190471	47618	1.38	0.243
Temperature. Packaging materials. Chemical treatment	2	283407	141704	4.10	0.018
Temperature. Chemical treatment. Storage time	2	147020	73510	2.13	0.122
Temperature. Packaging materials. Storage time	4	209888	52472	1.52	0.198
Packaging materials. Chemical treatment. Storage time	4	12581	3145	0.09	0.985
Temperature. Packaging materials. Chemical treatment. Storage time	4	228805	57201	1.66	0.162
Residual	72	6216146	34534		
Total	107	17290317			

Table A181. Malvidin 3-glucoside concentration of Experiment 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	144.68	144.68	28.62	<.001
Chemical treatment	1	146.04	146.04	28.89	<.001
Packaging materials	2	110.29	55.15	10.91	<.001
Storage time	2	716.87	358.44	70.91	<.001
Temperature.Chemical treatment.	1	0.10	0.10	0.02	0.889
Temperature.Packaging materials	2	4.10	2.05	0.41	0.667
Packaging materials.Chemical treatment	2	429.53	214.77	42.49	<.001
Temperature. Storage time	2	22.58	11.29	2.23	0.110
Chemical treatment. Storage time	2	61.20	30.60	6.05	0.003
Packaging materials. Storage time	4	18.09	4.52	0.89	0.468
Temperature. Packaging materials. Chemical treatment	2	44.64	22.32	4.42	0.013
Temperature. Chemical treatment. Storage time	2	15.58	7.79	1.54	0.217
Temperature. Packaging materials. Storage time	4	38.74	9.68	1.92	0.110
Packaging materials. Chemical treatment. Storage time	4	41.53	10.38	2.05	0.089
Temperature. Packaging materials. Chemical treatment. Storage time	4	95.51	23.88	4.72	0.001
Residual	72	909.84	5.06		
Total	107	2799.33			

APPENDIX B.

VISUAL APPEARANCE OF STORED LITCHI FRUIT DURING STORAGE TIME

CHAPTER 4

DAY 1

	N/A	N/A
5°	8°C	10°C

	
13°C	20°C

DAY 6

	N/A	N/A
5°	8°C	10°C

	
13°C	20°C

DAY 10

	N/A	N/A
5°	8°C	10°C

	
13°C	20°C

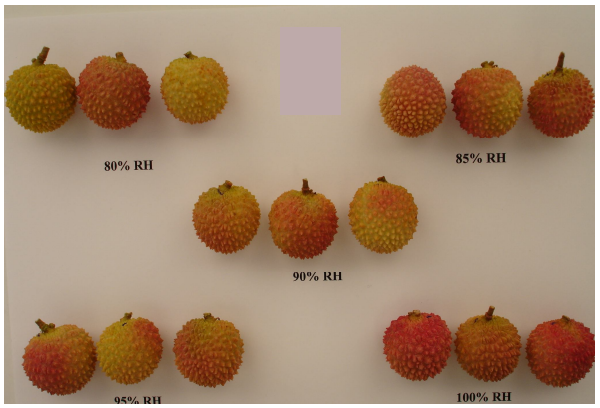
DAY 13

	N/A	N/A
5°	8°C	10°C

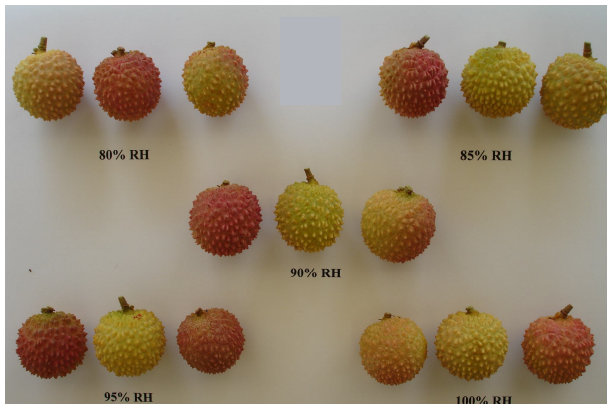
	
13°C	20°C

CHAPTER 5

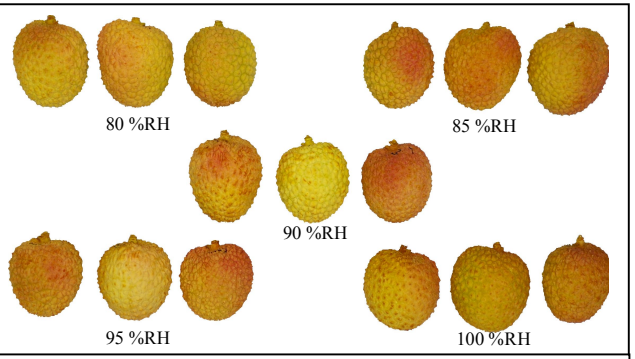
DAY 0



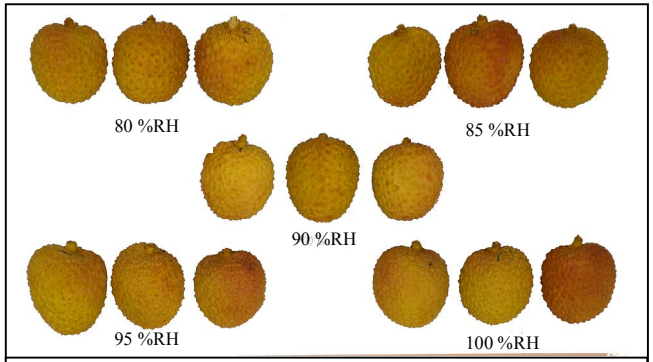
CV. Kom: 5°C



CV. Kom: 13°C

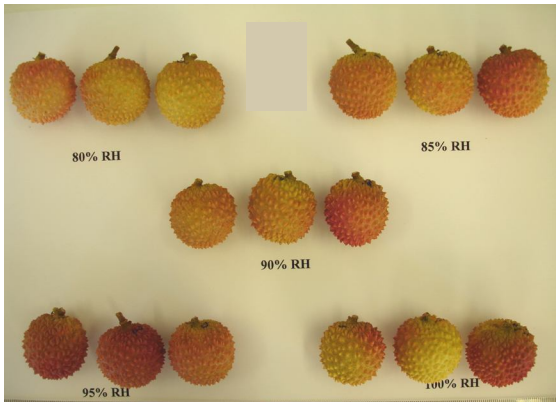


CV. Mauritius: 5°C



CV. Mauritius: 13°C

DAY 1



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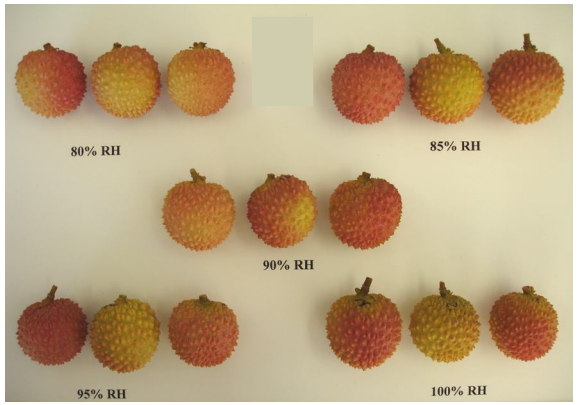
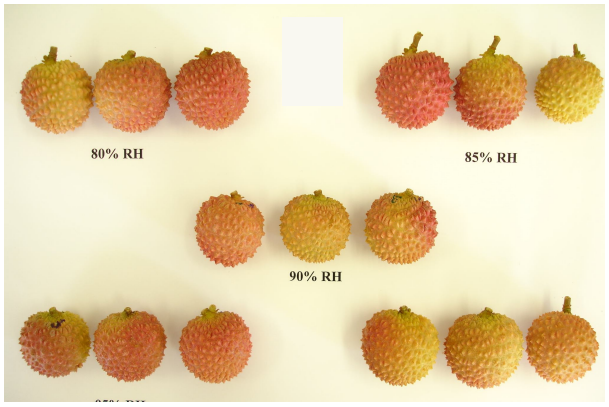


CV. Kom: 13°C

CV. Mauritius: 5°C

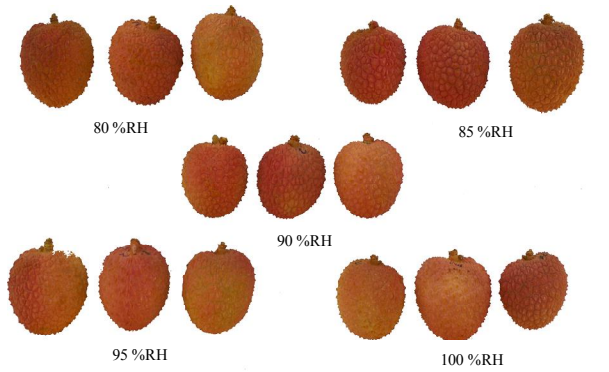
CV. Mauritius: 13°C

DAY 3

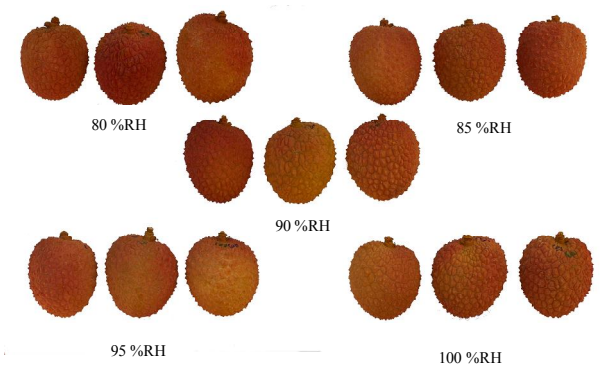


CV. Kom: 5°C

CV. Kom: 5°C

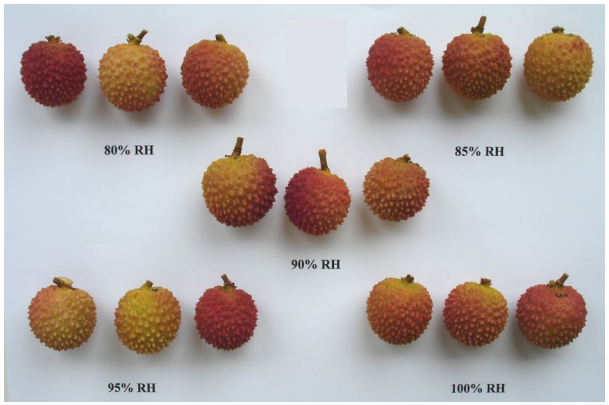


CV. Mauritius: 5°C

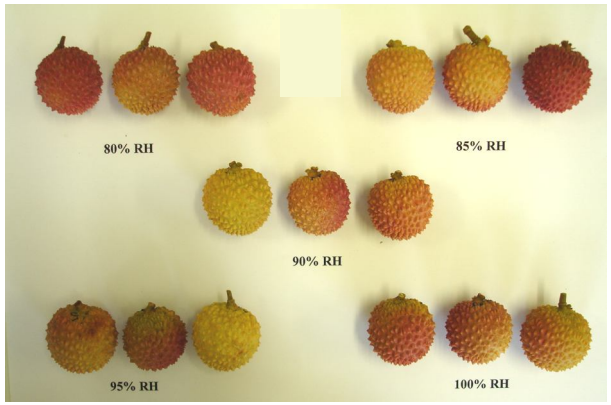


CV. Mauritius: 13°C

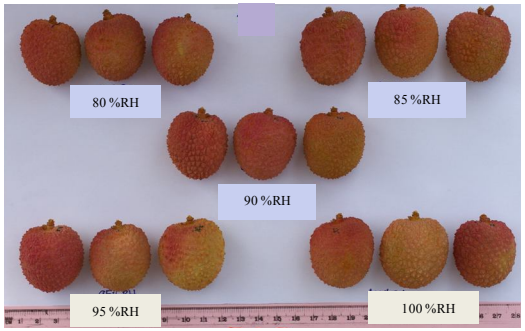
DAY 6



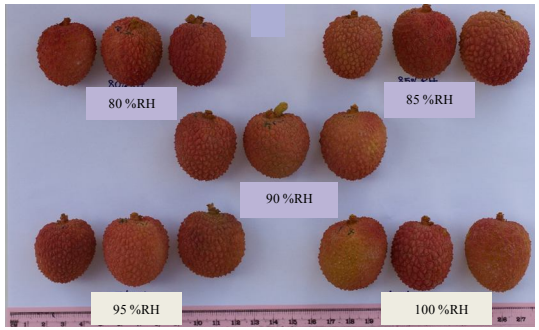
CV. Kom: 5°C



CV. Kom: 13°C

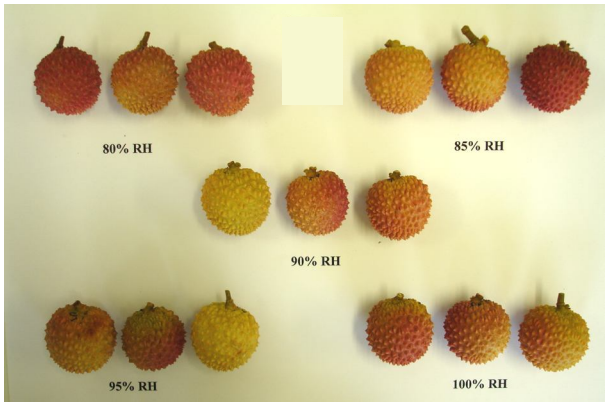


CV. Mauritius: 5°C

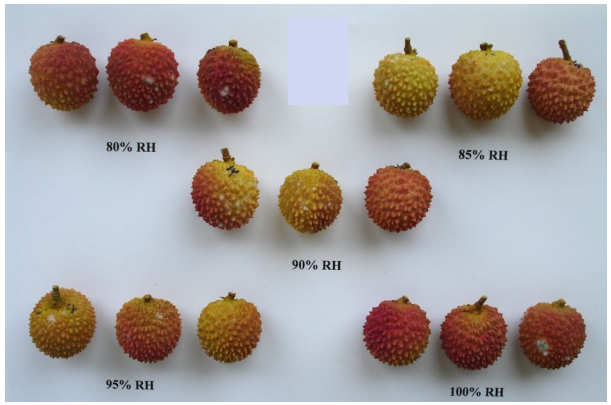


CV. Mauritius: 13°C

DAY 9

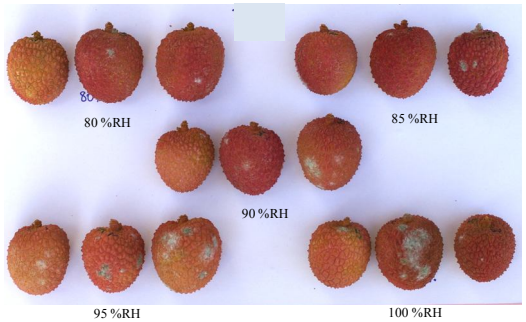


CV. Kom: 5°C

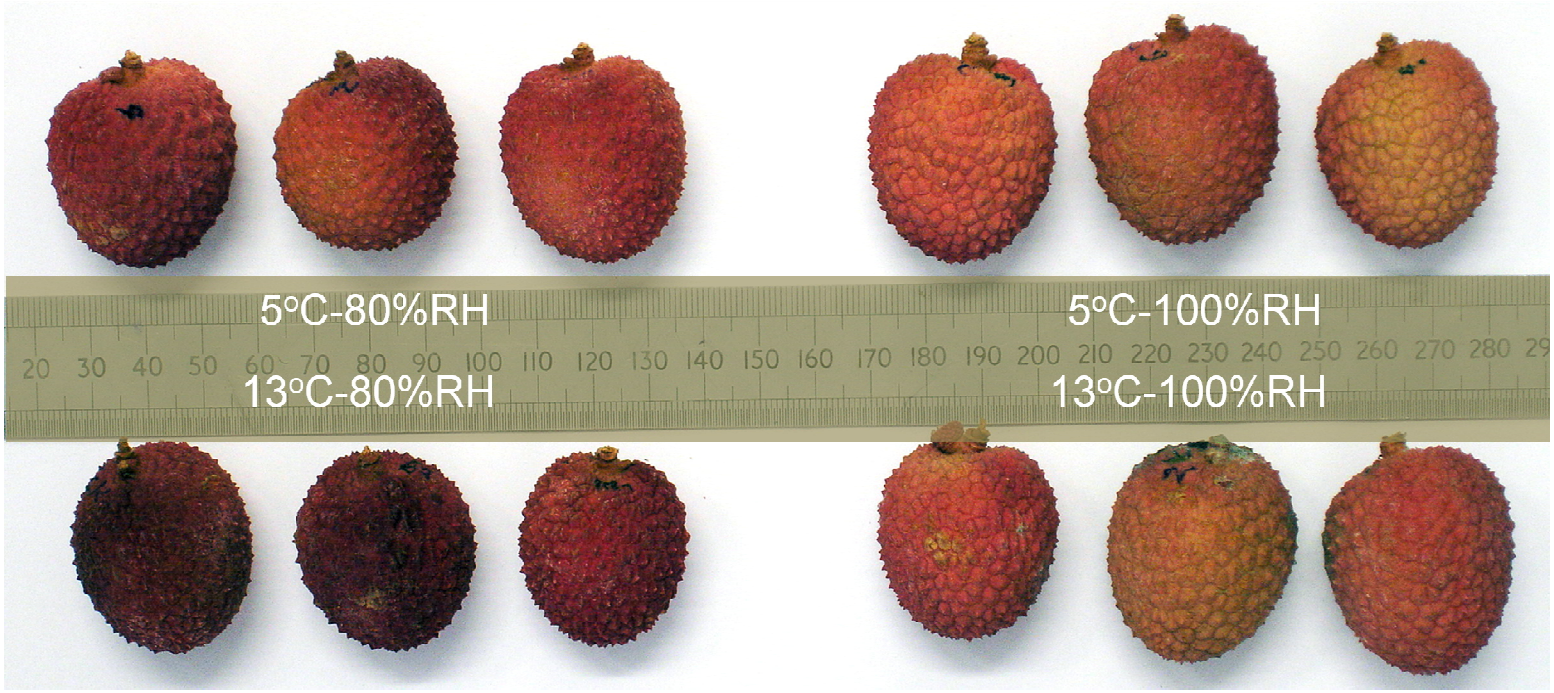


CV. Kom: 13°C

CV. Mauritius: 5°C



CV. Mauritius: 13°C

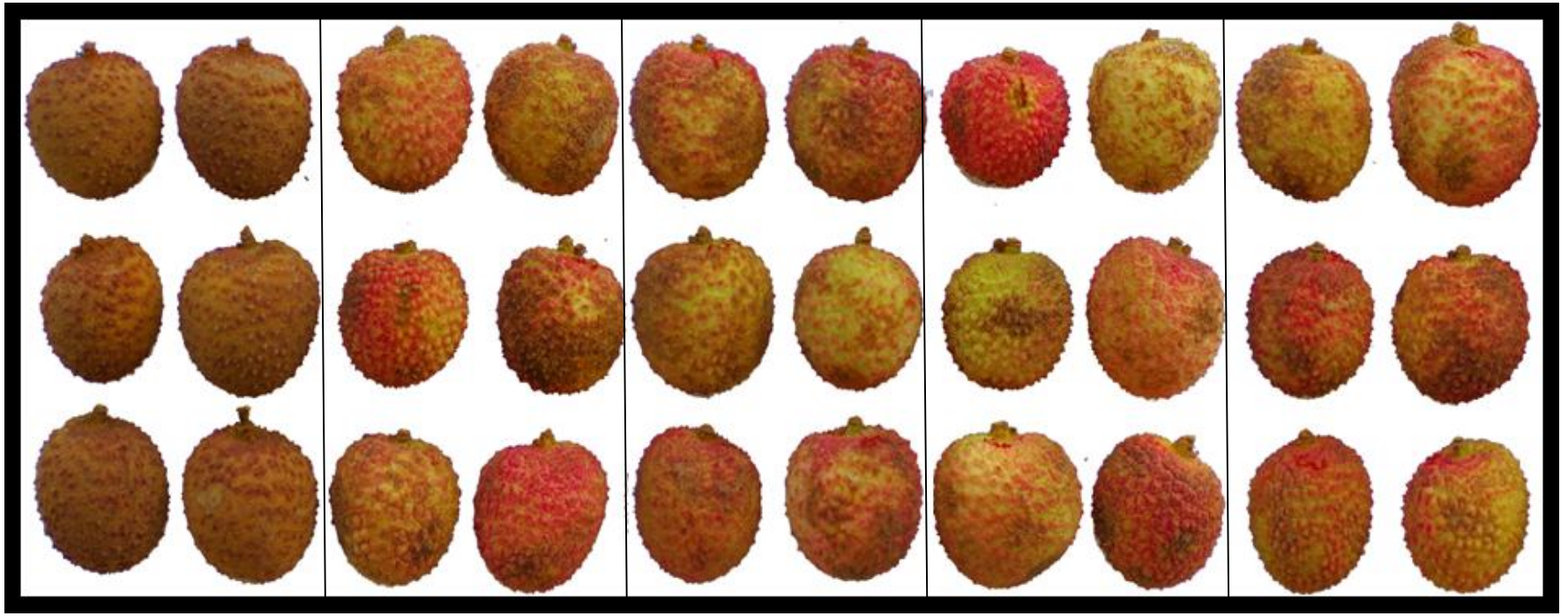


Litchi cv. Mauritius after 9 days treated at 5 or 13°C with 80 or 100 %RH

CHAPTER 6

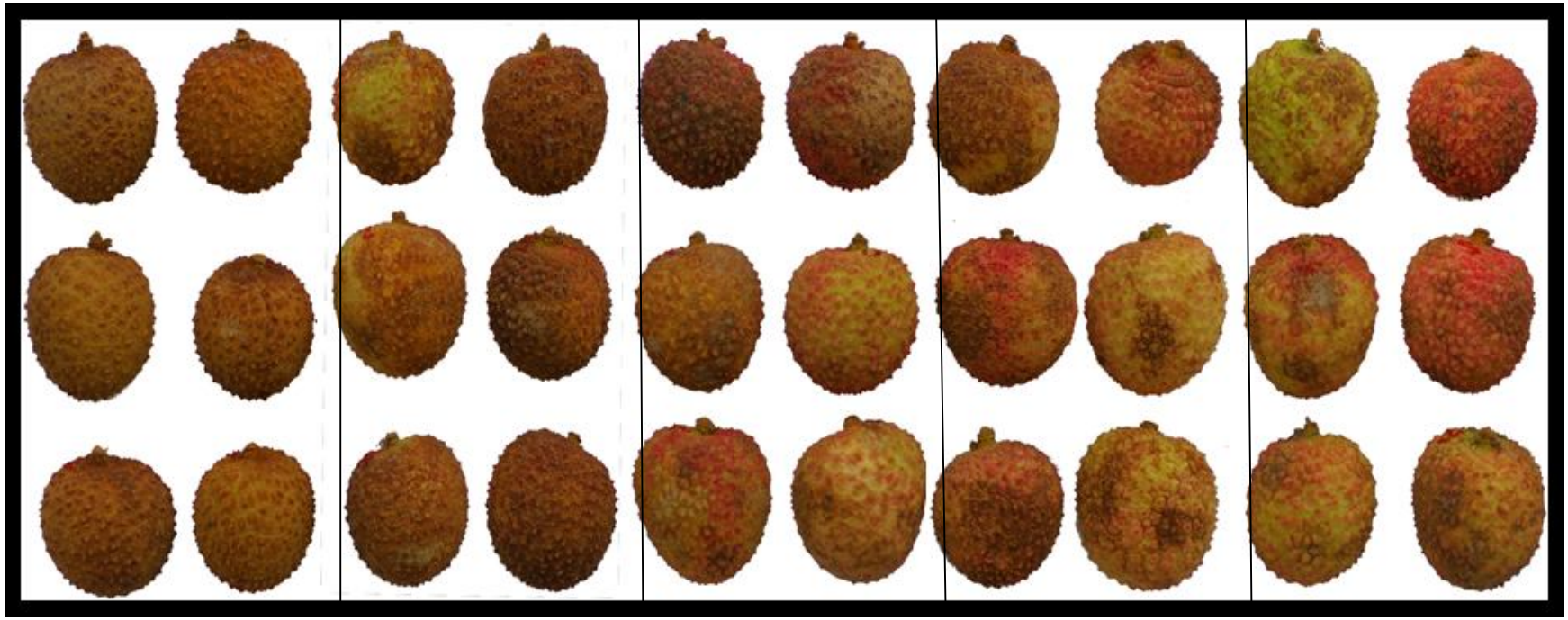
DAY 0

DAY 4



Unwrapped Fruit **NatureFlex™ NVS** **Perforated** **Cellophane™ WS** **PropaFresh™ PFAM**

DAY 9



Unwrapped Fruit **NatureFlex™ NVS** **Perforated** **Cellophane™ WS** **PropaFresh™ PFAM**

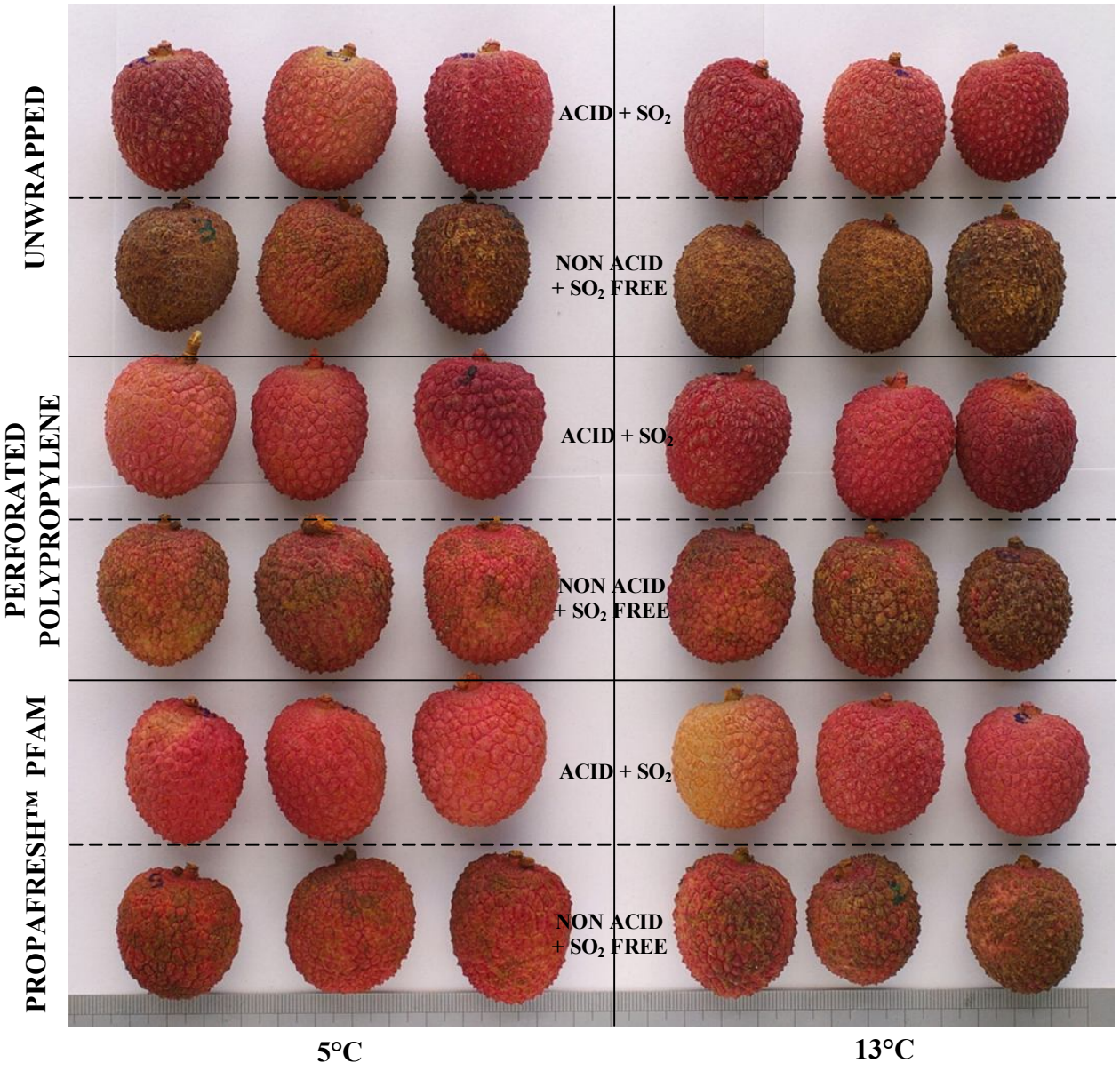
CHAPTER 7

DAY 0

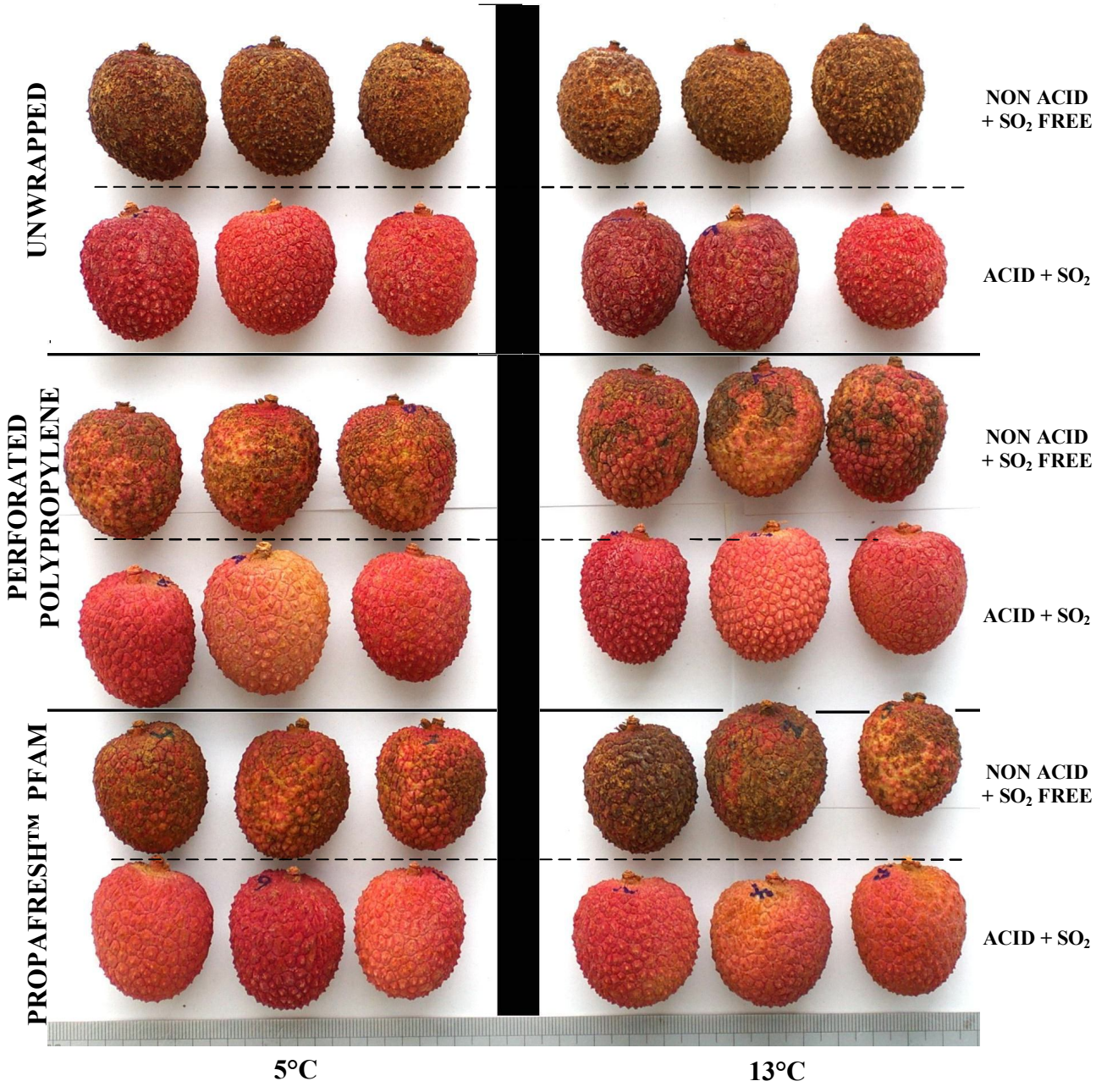
NON ACID TREATED + SO_2 FREE FRUIT

ACID TREATED + SO_2 FUMIGATED FRUIT

DAY 3


UNWRAPPED
PERFORATED POLYPROPYLENE
PROPAFRESH™ PFAM
ACID + SO2
NON ACID + SO2 FREE
ACID + SO2
NON ACID + SO2 FREE
ACID + SO2
NON ACID + SO2 FREE
5°C
13°C

DAY 7


UNWRAPPED
PERFORATED POLYPROPYLENE
PROPAFRESH™ PFAM
NON ACID + SO_2 FREE
ACID + SO_2
NON ACID + SO_2 FREE
ACID + SO_2
NON ACID + SO_2 FREE
ACID + SO_2
5°C
13°C

DAY 11

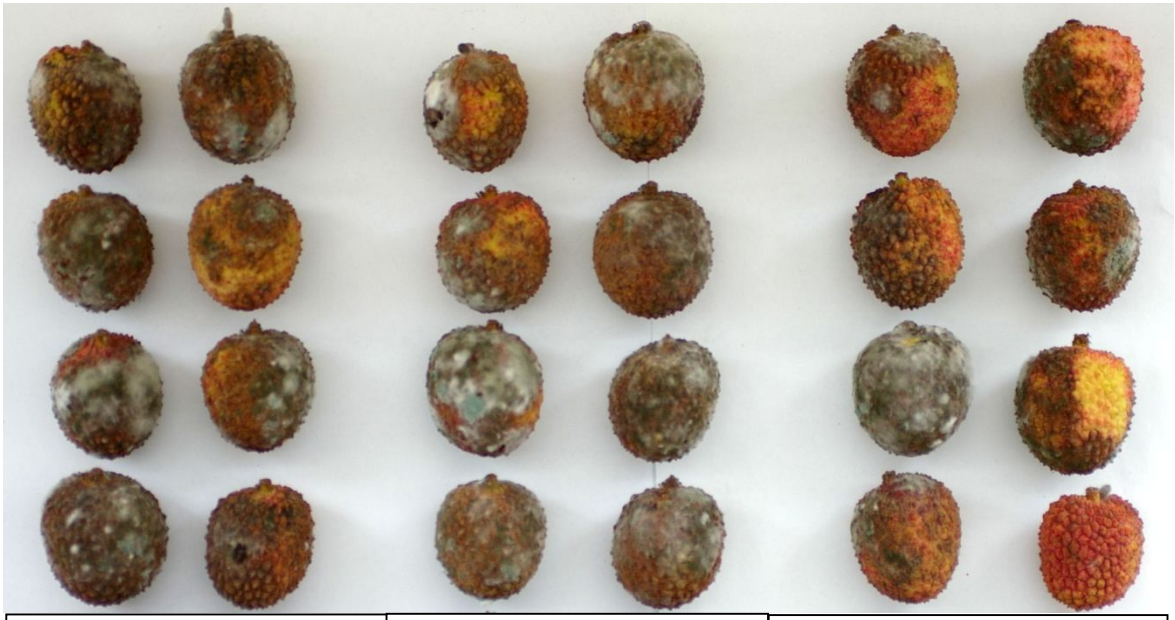

NON ACID + SO_2 FREE
ACID + SO_2
5°C
13°C
5°C
13°C
UNWRAPPED
PERFORATED POLYPROPYLENE
PROPAFRESH™ PFAM

COLOUR OF OUTER AND INNER PERICARP

ACID + SO_2 TREATED FRUIT PERICARP

NON ACID + SO_2 FREE TREATED FRUIT PERICARP

EFFECT OF ETHYLENE BLOCKER AND SCRUBER ON STORED LITCHI FRUIT AT 13°C FOR 11 DAYS



CONTROL	1-MCP	Pd

APPENDIX C.

POSTHARVEST DISEASE IN LITCHI FRUIT

POSTHARVEST DISEASE IN LITCHI FRUIT

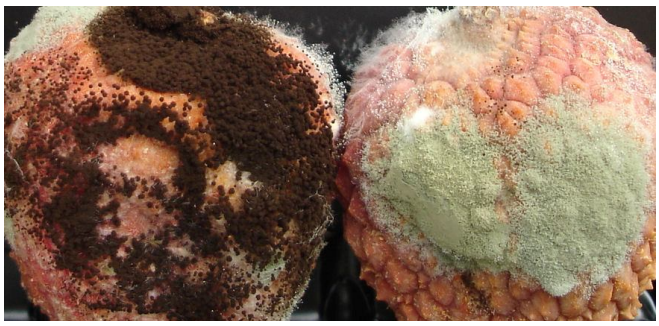
Apart from pericarp browning, postharvest rot is one of the major problems during litchi distribution which impacts fruit value. Although a wide range of fungi has been reported as a dominant pathogen in stored litchi fruit (Table 2.6), *Penicillium* spp., *Phomopsis* sp., *Alternaria* sp., *Botryodiplodia* sp. and *Fusarium* spp. are mostly detected. However, *Penicillium* spp. was recorded as main specie in both SO_2 fumigated alone (Jacobs and Korsten, 2004) or acid treated and SO_2 fumigated fruit (Lichter *et al*., 2004).

The decay found in litchi fruit in current study was recorded in this appendix. However, the pathogen has not been completely isolated and the pathogen specie, hence, has not been reported.

Day 13: 13°C

Day 13: 20°C

Day 16: 20°C



Day 21: 20°C

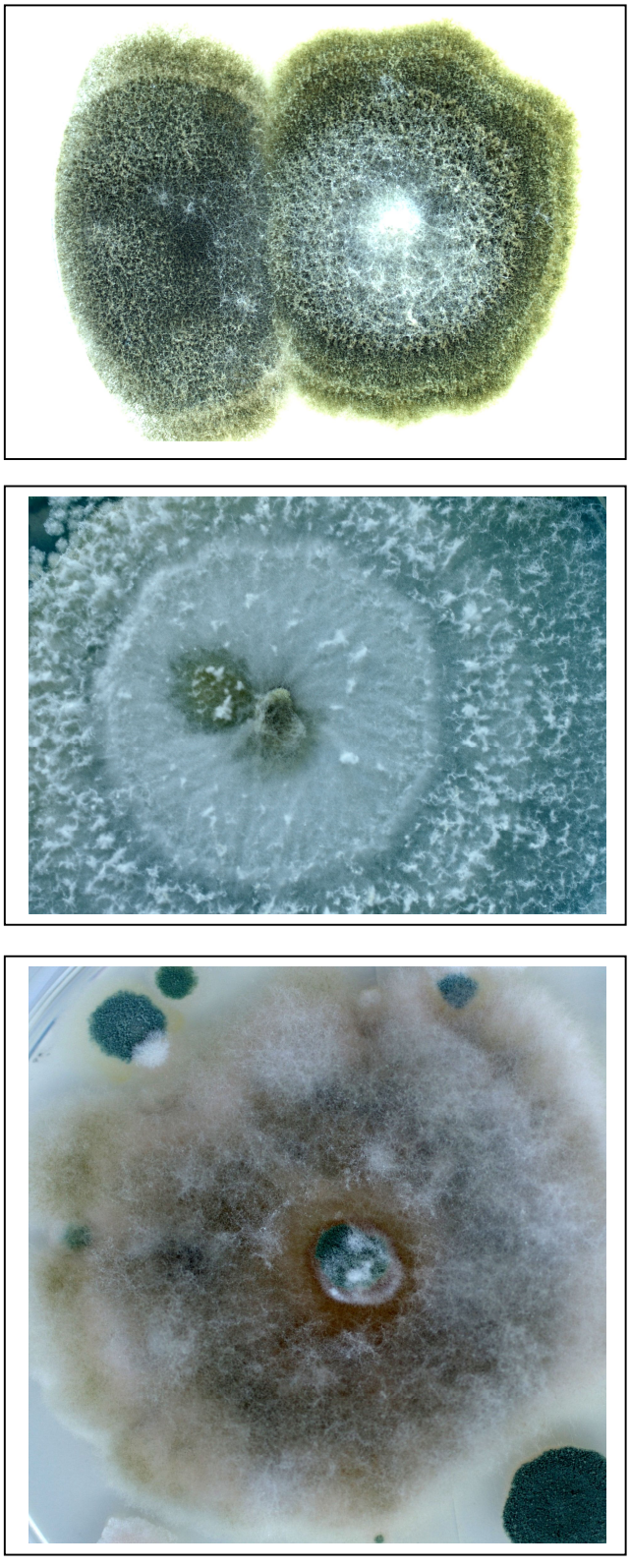
cv. KOM: Day 14: 13°C 100 %RH

cv. Mauritius: Day 14: 13°C 100 %RH

Fungi found in stored litchi fruit after 30 days storage

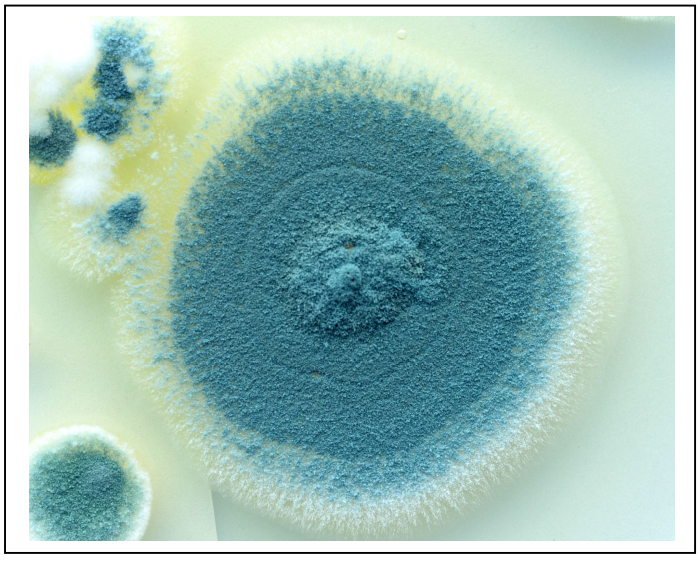
	
Non acid + non SO_2 treated Day 30: 13°C	Acid + SO_2 treated Day 30: 13°C

Fungi in non acid and non SO_2 treated fruit after 30 days storage



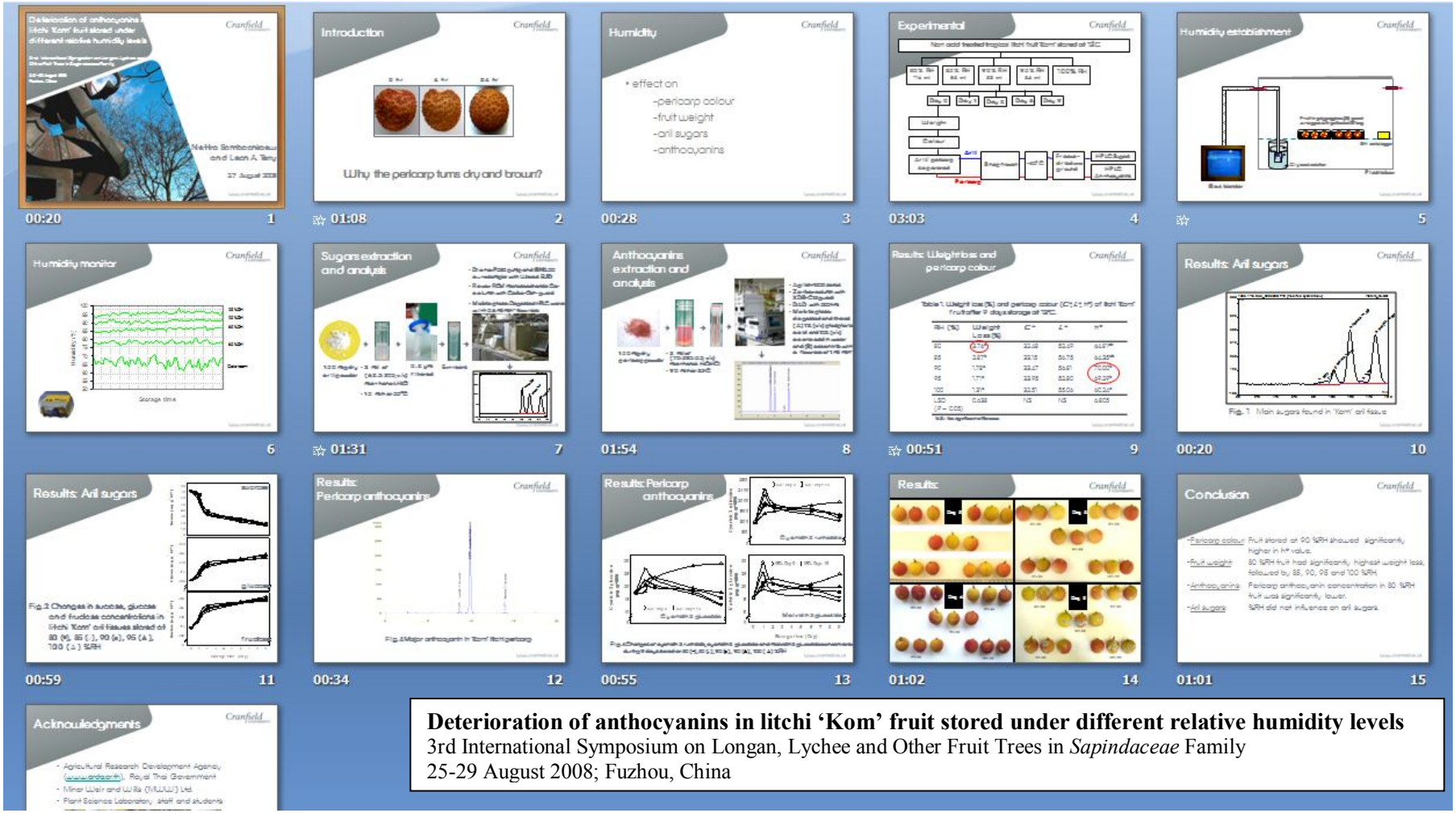
Fungi in acid and SO_2 treated fruit after 30 days storage

Fungi in both treatment fruit after 30 days storage



APPENDIX D.

PUBLICATIONS



Deterioration of anthocyanins in litchi 'Kom' fruit stored under different relative humidity levels
3rd International Symposium on Longan, Lychee and Other Fruit Trees in *Sapindaceae* Family
25-29 August 2008; Fuzhou, China

Deterioration of anthocyanins in litchi 'Kom' fruit stored under different relative humidity levels

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Abstract

The effects of relative humidity (RH) on litchi fruit quality have not yet been fully defined. The aim of this study was to detail the changes in physiology, non-structural carbohydrates (NSCs), and individual anthocyanin concentrations in imported litchi fruit held at various RH levels. Litchi 'Kom' fruit, imported from Thailand, were stored at 80, 85, 90, 95 or 100 %RH using different concentrations of glycerol in deionised water, at 13°C for 9 days. Fruit (n = 90) were individually measured for weight loss (%), hue (h°) and lightness (L^*) values. Freeze-dried aril and pericarp tissue were individually extracted and analysed for NSCs and anthocyanins, respectively.

Disease, mainly caused by *Penicillium* spp., was observed after 6 days storage which increased disease severity. Weight loss of fruit stored at 80 %RH was significantly higher than at other RH levels, whilst 90 %RH-stored fruit had a significantly higher h° over 9 days. The main NSCs in aril tissue were fructose (345.73 mg g^{-1} dry weight (DW)), glucose (329.08 mg g^{-1} DW) and sucrose (31.17 mg g^{-1} DW). There were no significant differences in NSCs concentration according to RH treatment. Generally, fructose and glucose concentrations increased during 9 days storage, whereas sucrose levels declined. The principal anthocyanins in pericarp tissue were cyanidin 3 rutinoside (1635.52 µg g^{-1} DW), cyanidin 3 glucoside (19.91 µg g^{-1} DW) and malvidin 3 glucoside (15.03 µg g^{-1} DW). All anthocyanins concentrations decreased after 9 days storage except fruit stored at 100 %RH which increased. Anthocyanin levels from 80 %RH-treated fruit were significantly lower than litchi held under higher RH conditions.

Keywords: cyanidin, lychee, malvidin, rutinoside, sugars

Introduction

Litchi chinensis Sonn. is a subtropical fruit with an attractive reddish-coloured pericarp and white semi-translucent aril. Although this fruit has been supplied and consumed world-wide for decades, substantial postharvest disorders such as fungal rots, pericarp browning and dehydration (Underhill *et al.*, 1997, Zhang *et al.*, 2001) are still the major problems facing growers and retailers.

Low temperature can slow down fruit metabolism, and pathogen growth. RH also plays an important role in determining postharvest life of litchi. Jiang & Fu (1999) and Kaewchana *et al.* (2006) demonstrated that RH influences pericarp browning of litchi 'Huaizhi' and 'Hong Huay' fruit, respectively. Both studies reported that litchi stored at 90 %RH showed the least change in a^* and L^* values and the smallest decrease in total anthocyanin and total phenolic content. Moreover, the slowest increase in polyphenol

Deterioration of anthocyanins in litchi 'Kom' fruit stored under different relative humidity levels
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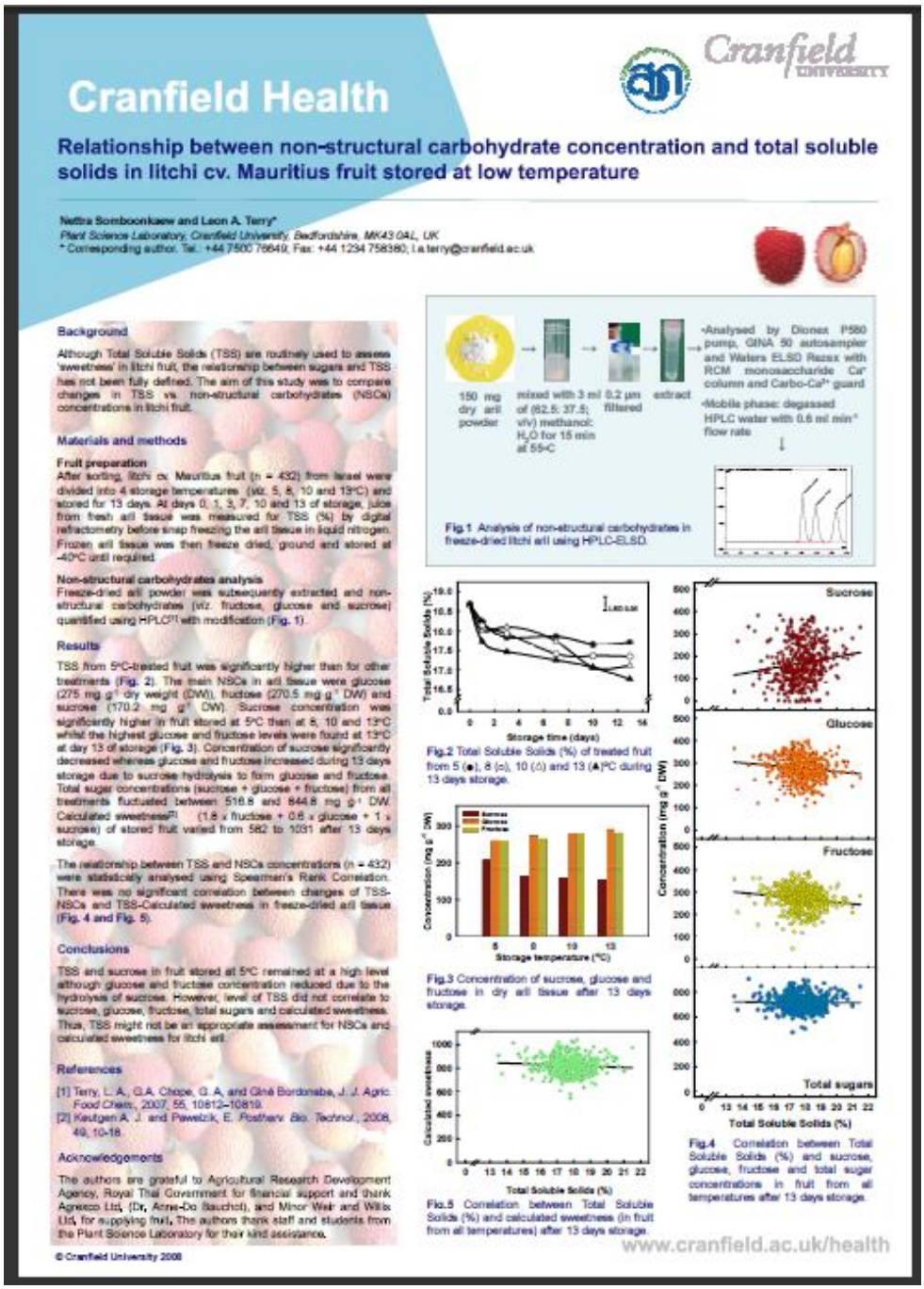

Cranfield Health

Relationship between non-structural carbohydrate concentration and total soluble solids in litchi cv. Mauritius fruit stored at low temperature

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Background

Although Total Soluble Solids (TSS) are routinely used to assess 'sweetness' in litchi fruit, the relationship between sugars and TSS has not been fully defined. The aim of this study was to compare changes in TSS vs. non-structural carbohydrates (NSCs) concentrations in litchi fruit.

Materials and methods

Fruit preparation
After sorting, litchi cv. Mauritius fruit (n = 432) from Israel were divided into 4 storage temperatures (viz. 5, 8, 10 and 13°C) and stored for 13 days. At days 0, 1, 3, 7, 10 and 13 of storage, juice from fresh aril tissue was measured for TSS (%) by digital refractometry before snap freezing the aril tissue in liquid nitrogen. Frozen aril tissue was then freeze dried, ground and stored at -40°C until required.

Non-structural carbohydrates analysis
Freeze-dried aril powder was subsequently extracted and non-structural carbohydrates (viz. fructose, glucose and sucrose) quantified using HPLC[1] with modification (Fig. 1).

Results

TSS from 5°C-treated fruit was significantly higher than for other treatments (Fig. 2). The main NSCs in aril tissue were glucose (275 mg g^{-1} dry weight (DW)), fructose (270.5 mg g^{-1} DW) and sucrose (170.2 mg g^{-1} DW). Sucrose concentration was significantly higher in fruit stored at 5°C than at 8, 10 and 13°C whilst the highest glucose and fructose levels were found at 13°C at day 13 of storage (Fig. 3). Concentration of sucrose significantly decreased whereas glucose and fructose increased during 13 days storage due to sucrose hydrolysis to form glucose and fructose. Total sugar concentrations (sucrose + glucose + fructose) from all treatments fluctuated between 516.8 and 844.8 mg g^{-1} DW. Calculated sweetness[2] (1.8 x fructose + 0.8 x glucose + 1 x sucrose) of stored fruit varied from 582 to 1031 after 13 days storage.

The relationship between TSS and NSCs concentrations (n = 432) were statistically analysed using Spearman's Rank Correlation. There was no significant correlation between changes of TSS-NSCs and TSS-Calculated sweetness in freeze-dried aril tissue (Fig. 4 and Fig. 5).

Conclusions

TSS and sucrose in fruit stored at 5°C remained at a high level although glucose and fructose concentration reduced due to the hydrolysis of sucrose. However, level of TSS did not correlate to sucrose, glucose, fructose, total sugars and calculated sweetness. Thus, TSS might not be an appropriate assessment for NSCs and calculated sweetness for litchi aril.

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[2] Keutgen A. J. and Pawelzik, E. Postharv. Bio. Technol., 2008, 49, 10-18.

Acknowledgements

The authors are grateful to Agricultural Research Development Agency, Royal Thai Government for financial support and thank Agrexco Ltd, (Dr. Anne-Do Bauchot), and Minor Weir and Willis Ltd, for supplying fruit. The authors thank staff and students from the Plant Science Laboratory for their kind assistance.

Fig.1 Analysis of non-structural carbohydrates in freeze-dried litchi aril using HPLC-ELSD.

Fig.2 Total Soluble Solids (%) of treated fruit from 5 (●), 8 (○), 10 (△) and 13 (▲)°C during 13 days storage.

Fig.3 Concentration of sucrose, glucose and fructose in dry aril tissue after 13 days storage.

Fig.4 Correlation between Total Soluble Solids (%) and sucrose, glucose, fructose and total sugar concentrations in fruit from all temperatures after 13 days storage.

Fig.5 Correlation between Total Soluble Solids (%) and calculated sweetness (in fruit from all temperatures) after 13 days storage.

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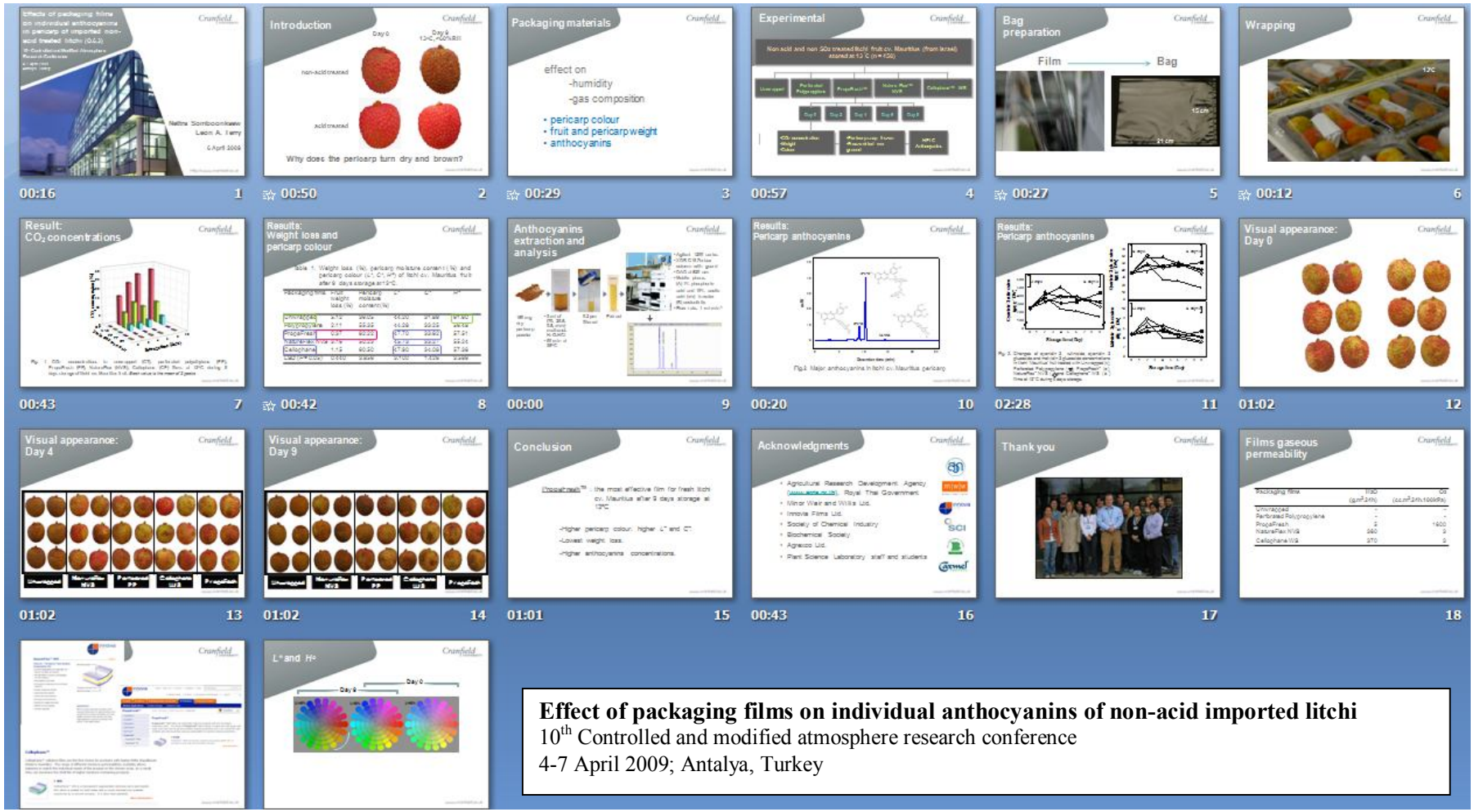
Relationship between non-structural carbohydrate concentration and total soluble solids in litchi cv. Mauritius fruit stored at low temperature
Postharvest Unlimited
4-7 November 2008; Potsdam, Berlin, Germany



Effect of packaging films on individual anthocyanins of non-acid imported litchi
10th Controlled and modified atmosphere research conference
4-7 April 2009; Antalya, Turkey

Effect of packaging films on individual anthocyanins in pericarp of imported non-acid treated litchi

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Litchi fruit (*Litchi chinensis* Sonn) are mainly produced in Asian and African countries and typically exported some distance from the growing area to the destination. If fruit are not handled correctly after harvest, they rapidly lose their attractive colour, which decreases their market appeal. Although sophisticated packaging materials can be used to minimise postharvest changes of litchi fruit, no work has documented the effect of modified atmosphere packaging on individual anthocyanin contents in litchi pericarp. Hence, the aim of this study was to detail the changes in weight, colour, and individual anthocyanin concentrations in litchi pericarp using various packaging materials. Non-acid-treated cv. Mauritius fruit, imported from Israel, were packed using 4 different packaging films *viz.* micro-perforated polypropylene (PP), PropaFresh™ (PF), NatureFlex™ NVS (NVS), Cellophane™ (CP) and unpacked (control), and stored at 13°C for 9 days. CO_2 and C_2H_4 were determined from a single pack (n = 60) whilst fruit (n = 378) were individually measured for weight loss (%), lightness (L^*), chroma (colour intensity, C^*) and hue (h^o) values. Freeze-dried pericarp tissue was extracted and analysed for individual anthocyanin content. Concentration of CO_2 (%) and C_2H_4 (µL. L^{-1}) were significantly greater in CP packs during 9 days storage followed by NVS, PF and PP films, respectively. Weight loss of fruit stored in PF film was significantly lower than for other treatments. Unwrapped fruit had significantly higher hue values (less red in colour) followed by CP, PP, NVS and PF-wrapped fruit after 9 days storage. However, fruit with CP film showed significantly higher L^* and C^* values over 9 days. The major anthocyanins found in pericarp tissue were cyanidin 3-rutinoside (328.00 µg g^{-1} DW), cyanidin 3-glucoside (42.88 µg g^{-1} DW) and malvidin 3-glucoside (5.01 µg g^{-1} DW). Anthocyanin concentrations from litchi wrapped with PF were significantly higher than for other plastic films after 9 days storage indicating that this treatment was the best. Small amount of fungi was found on PP-wrapped pericarp after 9 days storage.

Keywords: *Litchi chinensis*, lychee, MAP, plastic.

Effect of packaging films on individual anthocyanins of non-acid imported litchi
10th Controlled and modified atmosphere research conference
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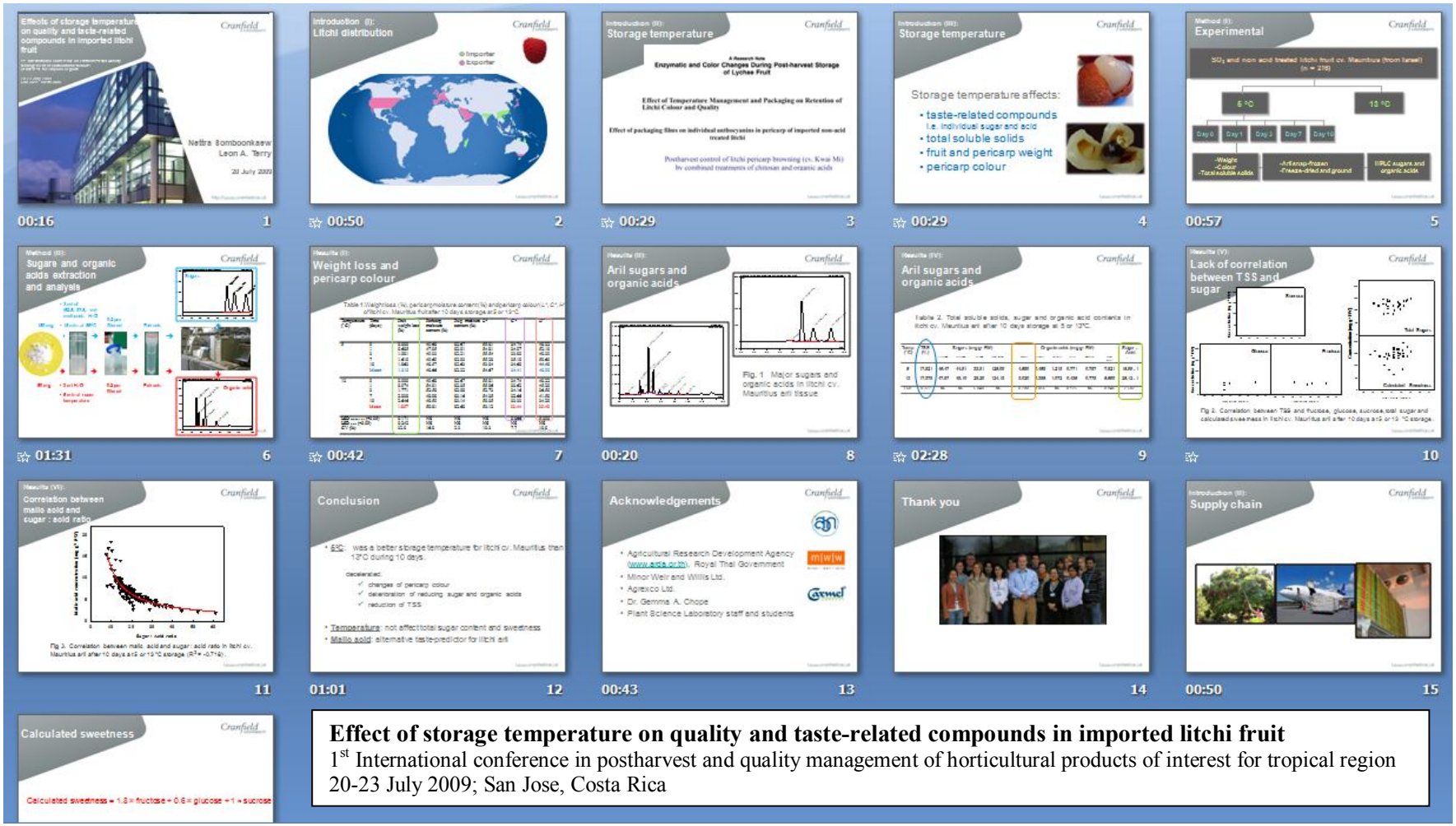

Effect of storage temperature on quality and taste-related compounds in imported litchi fruit
1st International conference in postharvest and quality management of horticultural products of interest for tropical region 20-23 July 2009; San Jose, Costa Rica

Effect of storage temperature on quality and taste-related compounds in imported litchi fruit

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Keywords: *Litchi chinensis*, lychee, organic acid, pericarp colour, sugar, TSS.

Abstract

Litchi (*Litchi chinensis* Sonn.) is a tropical to sub-tropical fruit which belongs to the *Sapindaceae* family. Although demand for fresh litchi in the world-wide market has increased moderately, trade in litchi fruit is still confronted by problems of weight loss and decay. This study aimed to detail the compositional biochemical changes in litchi aril tissues and changes fruit appearance as affected by different storage temperatures. Litchi 'Mauritius' fruit, imported from Israel, were stored at two different temperatures (5 and 13°C) for 10 days. Fruit (n = 150) were individually measured for weight loss, moisture content, total soluble solids (TSS) and skin colour (lightness (L^*), colour intensity (C^*) and redness (Hue, h°)). Freeze-dried aril tissue was extracted and analysed for individual sugar and organic acid contents. Fruit stored at 5°C had significantly lower weight loss (1.31%) and higher TSS (17.82%) after 10 days storage compared to those stored at 13°C. Pericarp colour of fruit stored at 5°C for 10 days was significantly higher in colour intensity (C^*) and redness (h°) values. The major sugars found in aril tissue were fructose (47.17 mg g^{-1} fresh weight (FW)), glucose (46.51 mg g^{-1} FW) and sucrose (31.01 mg g^{-1} FW). The level of TSS did not correlate with sucrose, glucose, fructose or total sugar concentration. Malic acid (3.80 mg g^{-1} FW) was the most important acid in litchi aril followed by tartaric (1.15 mg g^{-1} FW), ascorbic (0.78 mg g^{-1} FW), citric (0.60 mg g^{-1} FW) and oxalic (0.43 mg g^{-1} FW) acids. Although storage temperature did not affect total sugar content in litchi fruit, aril organic acids were significantly higher (1.37 fold) in fruit stored at 5°C resulting in a lower sugar : acid ratio. Results indicated that storage at 5°C maintained better postharvest quality and taste-related compound litchi fruit compared to 13°C.

Effect of storage temperature on quality and taste-related compounds in imported litchi fruit
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Postharvest Biology and Technology 56 (2010) 246–253

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Physiological and biochemical profiles of imported litchi fruit under modified atmosphere packaging

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ARTICLE INFO

Article history:
Received 3 December 2009
Accepted 23 January 2010

Keywords:
Anthocyanin
Carbon dioxide
Ethylene
MAP

ABSTRACT

Although sophisticated packaging materials can be used to minimise postharvest changes of litchi fruit, no work has documented the effect of different modified atmosphere packaging on biochemical composition of litchi aril and pericarp tissue. Therefore, the aim of this study was to detail not only the changes in weight and colour, but also individual sugars, organic acids and anthocyanin concentrations using various packaging materials. Non-acid- and SO_2-free fruit cv. Mauritius, imported from Israel, were packed using four different packaging films *viz.* micro-perforated polypropylene (PP), PropaFresh™ PFAM (PF), NatureFlex™ NVS (NVS), Cellophane™ WS (WS) and unwrapped, and stored at 13 °C for 9 days. Concentrations of CO_2 and ethylene were greater in WS packs during storage followed by NVS, PF and PP films, respectively. Weight loss of fruit stored in PF film was lower than for other treatments. The PF treatment

Altered physiology and biochemistry of imported litchi fruit held under different vapor pressure deficits

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Abstract

The effects of vapor pressure deficit (VPD) on litchi fruit quality have not yet been fully defined. The aim of this study was to detail the changes in physiology, sugars, organic acids and individual anthocyanin concentrations in imported litchi fruit held at various controlled relative humidity (RH) and VPC levels. SO_2 fumigated (but non acid-treated) litchi imported from Thailand (cv. Kom) and from Israel (cv. Mauritius), were air freighted to the UK and then stored for 9 days at either 5 or 13°C to simulate shelf life conditions. Fruits were stored under a series of controlled RH conditions for the duration of the trial using different concentrations of glycerol in deonized water. Respiration rate and weight loss of both fruit lots were greater in litchi stored at 13°C and VPD of 0.274 kPa. At 5°C and VPD of 0 or 0.042 kPa, sugars and organic acids in aril and pericarp tissue and individual anthocyanins in pericarp were better maintained. This is the first piece of work that has systematically evaluated the effect of a series of VPDs on litchi fruit biochemistry such that implications for designing systems to better maintain visual appearance of imported litchi fruit are discussed.

Influence of temperature and packaging on physiological and biochemical profiles of imported litchi fruit

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ABSTRACT

The aim of this study was to detail the physiological and biochemical changes in non acid and acid treated litchi fruit stored in different packaging films under different storage temperatures. Litchi fruit cv. Mauritius treated with SO_2 and acid, and free from both SO_2 and acid, were imported from Israel and packed using two different packaging films *viz.* micro-perforated polypropylene or PropaFresh™ PFAM, or stored unwrapped, at 5 or 13°C for 11 days. Both CO_2 and ethylene concentrations were higher in PropaFresh™ PFAM films, but lower concentrations were recorded for acid treated fruit and for 5°C storage. Weight loss was least in acid treated fruit wrapped with PropaFresh™ PFAM at 5°C. Non acid treated fruit wrapped in PropaFresh™ PFAM had higher individual aril sugars and organic acids whilst acid treated fruit retained higher concentrations of anthocyanins. These results indicate that PropaFresh™ PFAM packaging at 5°C were the best storage conditions to maintain postharvest quality in both acid and non acid treated litchi fruit.

Keywords: acid treatment, anthocyanin, carbon dioxide, ethylene, MAP, organic acid, sugar

1. Introduction

Pericarp browning is one of the main problems in harvested litchi fruit. Browning can be induced by fruit maturity and senescence (Sharma, Ray & Rai, 1986; Huang & Wang, 1990), disease (Huang & Scott, 1985; Sivakumar, Arrebola & Korsten, 2008), ethylene exposure and heat and chilling injury (Wong, Jacobi &